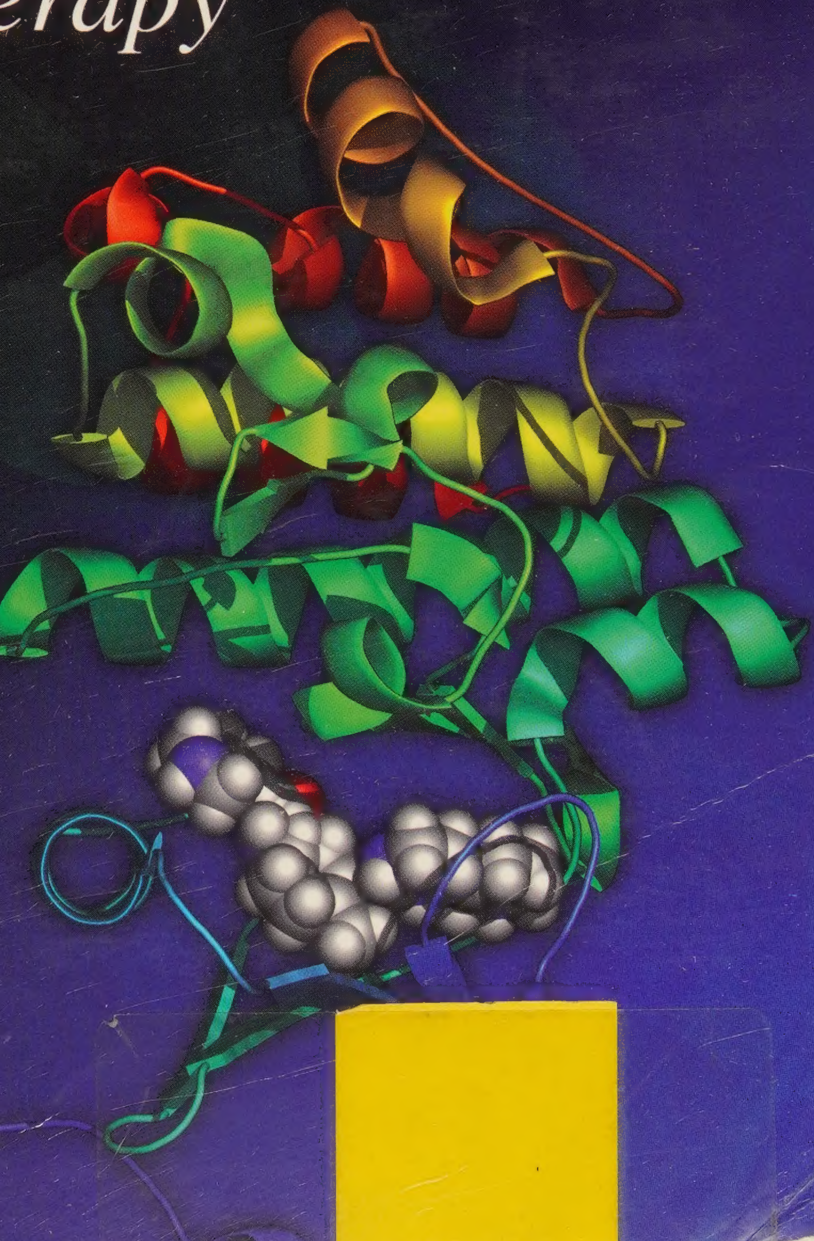
*0009133430*

# Principles of Pharmacology

## *The Pathophysiologic Basis of Drug Therapy*



David E. Golan
Armen H. Tashjian, Jr.
Ehrin J. Armstrong
Joshua M. Galanter
April Wang Armstrong
Ramy A. Arnaout
Harris S. Rose

Lippincott Williams & Wilkins

# Principles of Pharmacology:
## *Th[illegible] Pathophysiologic Basis of Dr[illegible] Therapy*

Digitized by the Internet Archive
in 2026

https://archive.org/details/principlesofphar0000unse_q2l4

CORK HEALTH SCIENCES BROOKFIELD
LIBRARY

# Principles of Pharmacology:
## *The Pathophysiologic Basis of Drug Therapy*

**David E. Golan, MD, PhD**
Editor-in-Chief

**Armen H. Tashjian, Jr., MD**
Deputy Editor

**Ehrin J. Armstrong, MD, MSc**
**Joshua M. Galanter, MD**
**April Wang Armstrong, MD**
**Ramy A. Arnaout, MD, DPhil**
**Harris S. Rose, MD**
Associate Editors

Lippincott Williams & Wilkins
A **Wolters Kluwer** Company
Philadelphia • Baltimore • New York • London
Buenos Aires • Hong Kong • Sydney • Tokyo

UNIVERSITY COLLEGE
Library
CORK

*Executive Editor: Betty Sun*
*Managing Editor: Amy Oravec*
*Marketing Manager: Joseph Schott*
*Production Editor: Jennifer Ajello*
*Designer: Doug Smock*
*Compositor: Circle Graphics*
*Printer: Quebecor World—Dubuque*

Copyright © 2005 Lippincott Williams & Wilkins

351 West Camden Street
Baltimore, MD 21201

530 Walnut Street
Philadelphia, PA 19106

All rights reserved. This book is protected by copyright. No part of this book may be reproduced in any form or by any means, including photocopying, or utilized by any information storage and retrieval system without written permission from the copyright owner.

The publisher, editors, and authors are not responsible (as a matter of product liability, negligence, or otherwise) for any injury resulting from any material contained herein. This publication contains information relating to general principles of medical care that should not be construed as specific instructions for individual patients. Manufacturers' product information and package inserts should be reviewed for current information, including drug indications, contraindications, dosages, route and duration of administration, and precautions.

*Printed in the United States of America*

**Library of Congress Cataloging-in-Publication Data**

Principles of pharmacology : the pathophysiologic basis of drug therapy / David E. Golan, editor-in-chief; Armen H. Tashjian, deputy editor; Ehrin J. Armstrong . . . [et al.], editors.
p. ; cm.
Includes index.
ISBN 0-7817-4678-7
1. Pharmacology. 2. Physiology, Pathological. I. Golan, David E.
[DNLM: 1. Chemical Actions. 2. Drug Therapy. 3. Pharmacology. QV 38 P9576 2004]
RM301.P65 2004
615′.1—dc22

2003060530

*The publishers have made every effort to trace the copyright holders for borrowed material. If they have inadvertently overlooked any, they will be pleased to make the necessary arrangements at the first opportunity.*

To purchase additional copies of this book, call our customer service department at **(800) 638-3030** or fax orders to **(301) 824-7390.** International customers should call **(301) 714-2324.**

***Visit Lippincott Williams & Wilkins on the Internet: http://www.LWW.com.*** Lippincott Williams & Wilkins customer service representatives are available from 8:30 am to 6:00 pm, EST.

05 06 07 08 09
3 4 5 6 7 8 9 10

Front Cover Caption:

The front cover image shows the molecular interaction between the catalytic domain of Abelson tyrosine kinase (Abl, ribbon representation) and an analogue of imatinib (space-filling representation). Imatinib is a highly selective inhibitor of Abl kinase, which is constitutively activated in chronic myelogenous leukemia (CML). Therapy with imatinib achieves a complete hematologic remission in a high percentage of patients with CML, with few adverse side effects. The development of resistance to imatinib is typically associated with a point mutation in Abl that disrupts imatinib binding. Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1FPU) by T Schindler, W Bornmann, P Pellicena, WT Miller, B Clarkson, and J Kuriyan (Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. Science 2000;289:1938–1942).

To the students of Harvard Medical School and
the Harvard School of Dental Medicine

# FOREWORD

Almost every practicing physician prescribes drugs; most write many prescriptions every day. The learning of pharmacology, the science that deals with the action and use of drugs, is among the most important steps in becoming a physician. Rather than reflexly ordering a medication to treat a specific symptom or disease, modern therapeutics requires an understanding of the underlying mechanism of action of a pharmacologic agent, how it influences and is influenced by the disease for which it is prescribed, and its capacity for causing both beneficial and harmful clinical effects.

Medical textbooks play a vital role in the education of students, residents, fellows, practicing physicians, and paramedical professionals. The large majority are written for anyone who will read—or preferably buy—them. As a consequence, they often provide a little for everyone but not enough for anyone. The sparsity of medical texts designed specifically for students leads faculty to spend countless hours preparing and duplicating voluminous lecture notes, and providing students with custom-designed "camels" (a camel is a cow created by a committee). Occasionally, these lecture notes are collated and published but these products have not been widely adopted. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy,* a collaborative project of Harvard Medical School Faculty and Students, represents a refreshing and innovative departure in the preparation of a medical text. The close collaboration between students and faculty experts, under the leadership of two distinguished scientist-educators, Drs. David E. Golan and Armen H. Tashjian, Jr., has resulted in this fine introductory text *specifically* designed to meet the needs of medical students. The superb illustrations greatly enhance the text and help to make complex concepts readily comprehensible.

Pharmacology is uniquely positioned among the biomedical sciences. It depends on and contributes to genetics, biochemistry, cell biology, organ physiology, and clinical medicine. Similarly, pharmacology occurs at a critical juncture in the medical curriculum, after completion of most of the basic science courses but before intense clinical experiences have begun. At this vital point in their education, most students are anxious to begin to apply their knowledge of science to clinical medicine. The clinical vignettes at the opening of every chapter demonstrate the critical importance of understanding pharmacologic principles to the solution of important clinical problems.

I predict with confidence that *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy* will play a vital role in the teaching and learning of pharmacology. Drs. Golan and Tashjian and their colleagues—both students and faculty—have made a significant and unique contribution in preparing this important book. Future generations of teachers and students, and ultimately the patients they will serve, will be indebted to them for this important contribution.

*Eugene Braunwald, MD*
*Distinguished Hersey Professor of Medicine*
*Harvard Medical School*
*Boston, Massachusetts*

# PREFACE

This book represents a new approach to the teaching of a first or second year medical school pharmacology course. The book, titled *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy,* departs from standard pharmacology textbooks in several ways. *Principles of Pharmacology* provides an understanding of drug action in the framework of human physiology, biochemistry, and pathophysiology. Each section of the book presents the pharmacology of a particular physiologic or biochemical system, such as the cardiovascular system or the inflammation cascade. Chapters within each section present the pharmacology of a particular aspect of that system, such as vascular tone or eicosanoids. Each chapter presents a clinical vignette illustrating the relevance of the system under consideration; then discusses the biochemistry, physiology, and pathophysiology of the system; and finally presents the drugs and drug classes that activate or inhibit the system by interacting with specific molecular and cellular targets. In this scheme, the therapeutic and adverse actions of drugs are understood in the framework of the drug's mechanism of action. The physiology, biochemistry, and pathophysiology are illustrated using clear and concise figures, and the pharmacology is depicted by displaying the targets in the system on which various drugs and drug classes act. Material from the clinical vignette is referenced at appropriate points in the discussion of the system. Contemporary directions in molecular and human pharmacology are introduced in chapters on modern methods of drug discovery and drug delivery and in a chapter on pharmacogenomics.

This approach has several advantages. We anticipate that students will use the text not only to learn pharmacology, but also to review essential aspects of physiology, biochemistry, and pathophysiology. Students will learn pharmacology in a conceptual framework that fosters mechanism-based learning rather than rote memorization, and that allows for ready incorporation of new drugs and drug classes into the student's fund of knowledge. Finally, students will learn pharmacology in a format that integrates the actions of drugs from the level of an individual molecular target to the level of the human patient.

The writing and editing of this textbook have employed a close collaboration among Harvard Medical School students and faculty in all aspects of book production, from student-faculty co-authorship of individual chapters to student-faculty editing of the final manuscript. In all, 43 HMS students and 39 HMS faculty have collaborated on the writing of the book's 52 chapters. This development plan has blended the enthusiasm and perspective of student authors with the experience and expertise of faculty authors, to provide a comprehensive and consistent presentation of modern, mechanism-based pharmacology.

David E. Golan, MD, PhD
Armen H. Tashjian, Jr., MD
Ehrin J. Armstrong, MD, MSc
Joshua M. Galanter, MD
April Wang Armstrong, MD
Ramy A. Arnaout, MD, DPhil
Harris S. Rose, MD

# ACKNOWLEDGMENTS

The editors are very grateful for support and assistance from a number of individuals. Valuable feedback on early versions of book chapters was provided by hundreds of medical and dental students at Harvard Medical School and the Harvard School of Dental Medicine and by several anonymous external reviewers. Salahadin Abdi, Rami Burstein, Ursula Kaiser, Gerald Pier, David Potter, Carl Rosow, Joachim Scholz, and Robert Shmerling kindly reviewed draft versions of one or more chapters. Christopher Cairo and Pallop Karnchanaphanurach expertly reviewed the chemical structures. Laura Green provided much-appreciated editorial assistance at critical stages in the preparation of the final manuscript. Marianne Lai helped to secure permissions for the use of borrowed material.

Rob Duckwall devoted tremendous energy, enthusiasm, creativity, and talent to the art generation and revision process. Working closely with him through the spring, summer, and fall of 2003 was one of the most rewarding aspects of this project.

Stuart Ferguson was a superb executive assistant to the authors and editors in every aspect of this project, including manuscript preparation and revision, art preparation and revision, chapter editing, and book production. His stamina, determination, organization, and good humor allowed the project to move forward at countless critical junctures. He kept track of authors and editors as they traveled to meetings and new positions across the US, and of manuscript drafts as they were exchanged among authors and editors. The magnitude of Stuart's contribution to this project cannot be captured fully in words.

Liz Allison was magnificent as our advisor, mentor, guide, cheerleader, and friend during the latter half of this project, including the critical book planning, editing, and production phases. We wish only that we had engaged her services sooner.

John Halamka, Liz Hess, and the Harvard University Provost's Office provided important advice, advocacy, and support at several critical junctures in this project.

We thank the editors and production managers at LWW, especially Betty Sun, Amy Oravec, and Jennifer Ajello, for their strong support and encouragement.

David Golan would also like to thank the many individuals whose support and understanding allowed him to maintain a teaching, research, and clinical presence while continuing to work on this project. Members of the Golan laboratory, the faculty and staff in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School, and the faculty and staff in the Hematology Division at Brigham and Women's Hospital and the Dana-Farber Cancer Institute were especially supportive and gracious throughout. Laura, Liza, and Sarah were constant sources of support and rejuvenation.

Armen Tashjian thanks Carol for her enthusiastic encouragement throughout the highs and lows of this project.

*Credit lines identifying the original source of a figure or table borrowed or adapted from copyrighted material, and acknowledging the use of non-copyrighted material, are gathered together in a list at the end of the book. We thank all of these sources for permission to use this material.*

# CONTRIBUTORS

**Ryan L. Albritton, MD**
Integrated Transitional Residency Program
John A. Burns School of Medicine
University of Hawai'i at Manoa
Honolulu, HI

**Seth L. Alper, MD, PhD**
Professor of Medicine, Harvard Medical School
Associate Chief, Molecular and Vascular Medicine Unit, Beth Israel Deaconess Medical Center
Renal Division, Beth Israel Deaconess Medical Center
Boston, MA

**April Wang Armstrong, MD**
Harvard Medical School
Boston, MA

**Ehrin J. Armstrong, MD, MSc**
Harvard Medical School
Boston, MA

**Sarah R. Armstrong, MS, DABT**
Senior Scientist
Cambridge Environmental Inc.
58 Charles St.
Cambridge, MA

**Ramy A. Arnaout, DPhil, MD**
Department of Pathology
Brigham and Women's Hospital
Boston, MA

**Mallar Bhattacharya, MD, MSc**
Harvard Medical School
Boston, MA

**Xandra O. Breakefield, PhD**
Professor of Neurology, Harvard Medical School
Geneticist (Neurology and Radiology), Molecular Neurogenetics Unit
Massachusetts General Hospital
Boston, MA

**David Lopes Cardozo, PhD**
Lecturer on Neurobiology
Department of Neurobiology
Harvard Medical School
Boston, MA

**Yeang H. Ch'ng, MS**
MD-PhD Program
Division of Medical Sciences, Neuroscience
Harvard Medical School
Boston, MA

**Michael S. Chang, MD**
Orthopaedic Surgery
University of Iowa Hospitals and Clinics
Iowa City, IA

**William W. Chin, MD**
Vice President
Lilly Research Laboratories
Eli Lilly & Company
Adjunct Professor of Medicine, Indiana University School of Medicine
Indianapolis, IN

**Janet Chou, MD**
Harvard Medical School
Boston, MA

**Vinh Chung, MD, MTh, MPharmSci**
Harvard Medical School
Boston, MA

**Jay Hwan Chyung**
MD-PhD Program
Harvard Medical School
Boston, MA

**David E. Clapham, MD, PhD**
Aldo R. Castañeda Professor of Cardiovascular Research
Investigator, Howard Hughes Medical Institute
Professor of Neurobiology, Harvard Medical School
Boston, MA

**Donald M. Coen, PhD**
Professor of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Boston, MA

**Scott M. Damrauer, MD**
Harvard Medical School
Boston, MA

**George D. Demetri, MD**
Associate Professor of Medicine, Harvard Medical School
Director, Center for Sarcoma and Bone Oncology
Department of Medical Oncology
Dana-Farber Cancer Institute
Boston, MA

**Robert G. Dluhy, MD**
Professor of Medicine
Associate Director, Division of Endocrinology, Diabetes and Hypertension
Department of Medicine
Harvard Medical School, Brigham and Women's Hospital
Boston, MA

**Jeffrey M. Drazen, MD**
Editor-in-Chief, The New England Journal of Medicine
Professor of Medicine, Harvard Medical School
Boston, MA

**David M. Dudzinski, JD**
Medical Student, Health Sciences and Technology Program
Harvard Medical School
Boston, MA

**Christopher H. Fanta, MD**
Associate Professor of Medicine, Harvard Medical School
Director, Partners Asthma Center
Pulmonary and Critical Care Division, Brigham and Women's Hospital
Boston, MA

**Daniel D. Federman, MD**
Carl W. Walter Distinguished Professor of Medicine and Medical Education
Harvard Medical School
Department of Medicine, Brigham and Women's Hospital
Boston, MA

**David A. Frank, MD, PhD**
Assistant Professor of Medicine, Harvard Medical School
Department of Medical Oncology
Dana-Farber Cancer Institute
Boston, MA

**Joshua M. Galanter, MD**
Department of Medicine
UCLA Medical Center
Los Angeles, CA

**David E. Golan, MD, PhD**
Professor of Biological Chemistry and Molecular Pharmacology, Professor of Medicine, Harvard Medical School
Scholar and Founding Member, The Academy at Harvard Medical School
Physician, Hematology Division, Brigham and Women's Hospital and Dana-Farber Cancer Institute
Co-Director, Harvard-M.I.T. MD-PhD Program
Department of Biological Chemistry and Molecular Pharmacology, Department of Medicine
Harvard Medical School
Boston, MA

**Laura C. Green, PhD, DABT**
Senior Scientist and President
Cambridge Environmental Inc.
Cambridge, MA
Lecturer
Biological Engineering Division
Massachusetts Institute of Technology
Cambridge, MA

**Edmund A. Griffin, Jr., MD, PhD**
Harvard Medical School
Boston, MA

**Robert S. Griffin**
MD-PhD Program
Harvard Medical School
Boston, MA

**Heidi Harbison, MD**
Harvard Medical School
Boston, MA

**Lloyd B. Klickstein, MD, PhD**
Assistant Professor of Medicine, Harvard Medical School
Director of Education, Division of Rheumatology, Immunology, and Allergy
Brigham and Women's Hospital
Boston, MA

**John C. LaMattina, MD**
Surgical Resident
Department of Surgery
Massachusetts General Hospital
Boston, MA

**Robert Langer, ScD**
Kenneth J. Germeshausen Professor of Chemical and Biomedical Engineering
Department of Chemical Engineering
Massachusetts Institute of Technology
Cambridge, MA

**Benjamin Leader, MD, PhD**
Resident in Emergency Medicine
Brown University Medical School
Rhode Island Hospital
Providence, RI

**Vivian Gonzalez Lefebre, MD**
Resident in Internal Medicine
Department of Medicine
Brigham and Women's Hospital
Boston, MA

**Allen S. Liu**
Medical Student
Harvard Medical School
Boston, MA

**Eng H. Lo, PhD**
Associate Professor, Program in Neuroscience
Harvard Medical School
Head, Neuroprotection Research Laboratory
Departments of Neurology and Radiology
Massachusetts General Hospital
Charlestown, MA

**Daniel H. Lowenstein, MD**
Professor of Neurology
Director, UCSF Epilepsy Center
Department of Neurology
University of California, San Francisco
San Francisco, CA

**Joren C. Madsen, MD, DPhil**
Associate Professor of Surgery, Harvard Medical School
Division of Cardiac Surgery
Massachusetts General Hospital
Boston, MA

**Kalpana L. Mani, MD**
Harvard Medical School
Boston, MA

**Janet F. McLaren, MD**
Resident in Obstetrics & Gynecology
Department of Obstetrics & Gynecology
Brigham and Women's Hospital, Massachusetts General Hospital
Boston, MA

**Thomas Michel, MD, PhD**
Associate Professor of Medicine (Biochemistry), Harvard Medical School
Chief of Cardiology, VA Boston Healthcare System
Physician, Cardiovascular Division, Brigham and Women's Hospital
Boston, MA

**Keith W. Miller, DPhil**
Edward Mallinckrodt Professor of Pharmacology
Departments of Anesthesia and of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Department of Anesthesia and Critical Care
Massachusetts General Hospital
Boston, MA

**Joshua D. Moss, MD**
Resident in Internal Medicine
Massachusetts General Hospital
Boston, MA

**Lorne W. Murray, MD**
Resident in Internal Medicine
Department of Medicine
Massachusetts General Hospital
Boston, MA

**Christopher W. Myers, MD, PhD**
Harvard Medical School
Boston, MA

**Martin G. Myers, Jr., MD, PhD**
Assistant Professor of Medicine, Harvard Medical School
Investigator, Joslin Diabetes Center
Boston, MA

**Dalia Shoretz Nagel, MD**
Harvard Medical School
Boston, MA

**Roy H. Perlis, MD**
Director, Psychopharmacology Consultation Service
Mood and Anxiety Disorder Institute
Massachusetts General Hospital
Instructor in Psychiatry, Harvard Medical School
Boston, MA

**Robert R. Rando, PhD**
Gustavus Adolphus Pfeiffer Professor
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Boston, MA

**Scott L. Rauch, MD**
Associate Professor of Psychiatry, Harvard Medical School
Associate Chief of Psychiatry for Neuroscience Research
Massachusetts General Hospital
Boston, MA

**Thomas P. Rocco, MD**
Assistant Professor of Medicine, Harvard Medical School
Director, Clinical Cardiology, VA Boston Healthcare System
Cardiovascular Division, Brigham and Women's Hospital
Boston, MA

**Harris S. Rose, MD**
Resident
Department of Surgery
Baylor College of Medicine
Houston, TX

**Robert H. Rubin, MD, FACP, FCCP**
Osborne Professor of Health Sciences and Technology and Professor of Medicine, Harvard Medical School
Director, Center for Experimental Pharmacology and Therapeutics, Harvard-M.I.T. Division of Health Sciences and Technology
Associate Director, Division of Infectious Disease, Brigham and Women's Hospital
Boston, MA

**Edward T. Ryan, MD, DTM&H**
Assistant Professor of Medicine, Harvard Medical School
Assistant Professor of Immunology and Infectious Diseases, Harvard School of Public Health
Director, Tropical and Geographic Medicine Center, Division of Infectious Diseases, Massachusetts General Hospital
Boston, MA

**Joan J. Ryoo, MD**
Harvard Medical School
Boston, MA

**Marvin Ryou, MD**
Resident in Internal Medicine
Department of Medicine
Brigham and Women's Hospital
Boston, MA

**Frank M. Sacks, MD**
Professor of Cardiovascular Disease Prevention, Harvard School of Public Health
Physician, Cardiovascular Division, Brigham and Women's Hospital
Nutrition Department
Harvard School of Public Health
Boston, MA

**Patrick K. Safo**
MD-PhD Program
Department of Neurobiology
Harvard Medical School
Boston, MA

**Ashish Sahasrabudhe, DMD**
Endodontic Resident
Department of Endodontics
New York University College of Dentistry
New York, NY

**John Samuelson, MD, PhD**
Professor
Department of Molecular and Cell Biology
Boston University Goldman School of Dental Medicine
Boston, MA

**Charles N. Serhan, PhD**
Simon Gelman Professor of Anesthesia (Biochemistry and Molecular Pharmacology), Harvard Medical School
Director, Center for Experimental Therapeutics and Reperfusion Injury
Senior Biochemist, Brigham and Women's Hospital
Brigham and Women's Hospital
Boston, MA

**Helen M. Shields, MD**
Associate Professor, Harvard Medical School
Department of Medicine
Division of Gastroenterology
Beth Israel Deaconess Medical Center
Boston, MA

**Aimee D. Shu, MD**
Resident in Internal Medicine
Department of Medicine
Brigham and Women's Hospital
Boston, MA

**Josef B. Simon, MD**
Department of Emergency Medicine
Harvard Affiliated Emergency Medicine Residency
Boston, MA

**Gary R. Strichartz, PhD**
Professor of Anaesthesia, Harvard Medical School
Director, Pain Research Center
Department of Anesthesiology, Perioperative and Pain Medicine
Brigham and Women's Hospital
Boston, MA

**Armen H. Tashjian, Jr., MD**
Professor of Biological Chemistry and Molecular Pharmacology, *emeritus*
Harvard Medical School
Professor of Toxicology, *emeritus*
Harvard School of Public Health
Department of Genetics and Complex Diseases
Harvard School of Public Health
Boston, MA

**Jeremy Tucker, MD**
Harvard Medical School
Boston, MA

**Patricia Tung, MPP**
Medical Student
Harvard Medical School
Boston, MA

**Timothy J. Turner, PhD**
Assistant Research Professor
Department of Neuroscience
Tufts University School of Medicine
Boston, MA

**Michael Tsan Ty, MD, MS**
Harvard-M.I.T. Division of Health Sciences and Technology
Boston, MA

**J. Wesley Ulm**
MD-PhD Program
Harvard-M.I.T. Division of Health Sciences and Technology
Program in Biological and Biomedical Sciences, Division of Medical Sciences, Harvard Graduate School of Arts and Sciences
Boston, MA

**Christopher T. Walsh, PhD**
Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Boston, MA

**Alan A. Wartenberg, MD, FACP, FASAM**
Assistant Professor of Medicine, Tufts University School of Medicine
Medical Director, Addiction Recovery Program
Faulkner Hospital
Boston, MA

**Ariel Weissmann, MD, MPH**
Harvard Medical School
Boston, MA

**Freddie M. Williams, MD**
Resident in Internal Medicine
Department of Medicine
Duke University Medical Center
Durham, NC

**Andrea S. Wolf, MD**
Clinical Fellow in Surgery
Department of Surgery
Massachusetts General Hospital
Boston, MA

**Clifford J. Woolf, MD, PhD**
Richard J. Kitz Professor of Anesthesia Research, Harvard Medical School
Neural Plasticity Research Group, Department of Anesthesia and Critical Care
Massachusetts General Hospital
Boston, MA

**Jacob Wouden, MD**
Transitional Intern
Boston University Program at Brockton Hospital, Brockton, MA
Diagnostic Radiology Resident
Harvard Program at Brigham and Women's Hospital
Boston, MA

**Deborah C. Yeh, MD**
Harvard Medical School
Boston, MA

**Robert W. Yeh, MD, MBA, MSc**
Resident in Internal Medicine
Department of Medicine
Massachusetts General Hospital
Boston, MA

# CONTENTS

Foreword vii

Preface ix

Acknowledgments xi

Contributors xiii

**SECTION I** FUNDAMENTAL PRINCIPLES OF PHARMACOLOGY. . . . . . 1

1 Drug-Receptor Interactions 3

2 Pharmacodynamics 17

3 Pharmacokinetics 27

4 Drug Metabolism 45

**SECTION II** PRINCIPLES OF NEUROPHARMACOLOGY. . . . . . 55

Section IIA Fundamental Principles of Neuropharmacology 57

5 Principles of Cellular Excitability and Electrochemical Transmission 59

6 Principles of Nervous System Physiology and Pharmacology 71

Section IIB Principles of Autonomic and Peripheral Nervous System Pharmacology 87

7 Cholinergic Pharmacology 89

8 Adrenergic Pharmacology 107

9 Local Anesthetic Pharmacology 121

Section IIC Principles of Central Nervous System Pharmacology 137

10 Pharmacology of Excitatory and Inhibitory Neurotransmission 139

11 Pharmacology of Dopaminergic Neurotransmission 161

12 Pharmacology of Affective Disorders 179

13 Pharmacology of Abnormal Electrical Neurotransmission in the Central Nervous System 193

14 General Anesthetic Pharmacology 205

15 Pharmacology of Analgesia 229

16 Pharmacology of Chemical Dependence and Addiction 247

SECTION III PRINCIPLES OF CARDIOVASCULAR PHARMACOLOGY . . . . . 265

17 Pharmacology of Cardiac Rhythm 267

18 Pharmacology of Cardiac Contractility 285

19 Pharmacology of Volume Regulation 299

20 Pharmacology of Vascular Tone 317

21 Pharmacology of Hemostasis and Thrombosis 335

22 Pharmacology of Cholesterol and Lipoprotein Metabolism 357

23 Integrative Cardiovascular Pharmacology: Hypertension, Ischemic Heart Disease, and Congestive Heart Failure 375

SECTION IV PRINCIPLES OF ENDOCRINE PHARMACOLOGY . . . . . 401

24 Pharmacology of the Hypothalamus and Pituitary Gland 403

25 Pharmacology of the Thyroid Gland 415

26 Pharmacology of the Adrenal Cortex 425

27 Pharmacology of Reproduction 441

28 Pharmacology of the Endocrine Pancreas 457

29 Pharmacology of Bone Mineral Homeostasis 473

SECTION V PRINCIPLES OF CHEMOTHERAPY . . . . . 491

30 Principles of Antimicrobial and Antineoplastic Pharmacology 493

31 Pharmacology of Nucleotide Synthesis 505

32 Pharmacology of DNA Replication and Mitosis 519

33 Pharmacology of Transcription and Translation 535

34 Pharmacology of Viral Replication 545

35 Pharmacology of the Bacterial Cell Wall 565

36 Pharmacology of Fungal Infections 579

37 Pharmacology of Parasitic Infections 587

38 Principles of Combination Chemotherapy 603

**SECTION VI** PRINCIPLES OF INFLAMMATION AND IMMUNE PHARMACOLOGY . . . . . 615

**39** Principles of Inflammation and the Immune System 617

**40** Pharmacology of Eicosanoids 627

**41** Histamine Pharmacology 647

**42** Pharmacology of Hematopoietic and Immune Cell Stimulation 655

**43** Pharmacology of Immunosuppression 667

**44** Integrative Inflammation Pharmacology: Peptic Ulcer Disease 683

**45** Integrative Inflammation Pharmacology: Asthma 695

**46** Integrative Inflammation Pharmacology: Gout 709

**SECTION VII** PRINCIPLES OF TOXICOLOGY . . . . . 717

**47** Principles of Toxicology 719

**SECTION VIII** CONTEMPORARY APPROACHES TO DRUG DISCOVERY, DEVELOPMENT AND DELIVERY . . . . . 745

**48** Drug Discovery and Development 747

**49** Protein-Based and Gene Therapies 757

**50** Drug Delivery Mechanisms 781

**SECTION IX** FUNDAMENTALS OF DRUG EVALUATION AND PHARMACOGENOMICS . . . . . 789

**51** New Drug Evaluation and Drug Regulation 791

**52** Pharmacogenomics 803

[illegible] **811**

[illegible] **815**

# Fundamental Principles of Pharmacology

# 1

# Drug-Receptor Interactions

Josef B. Simon and David E. Golan

**Introduction**
**Case**
**Conformation and Chemistry of Receptors and Ligands**
  Impact of Drug Binding on Receptor Conformation
  Membrane Effects on Drug-Receptor Interactions
**Major Types of Drug Receptors**
  Transmembrane Ion Channels
  Transmembrane G Protein-Coupled Receptors
  Transmembrane Receptors with Enzymatic Cytosolic Domains
    *Receptor Tyrosine Kinases*
    *Receptor Tyrosine Phosphatases*
    *Tyrosine Kinase-Associated Receptors*
    *Receptor Serine/Threonine Kinases*
    *Receptor Guanylyl Cyclases*
  Intracellular Receptors
**Processing of Signals Resulting from Drug-Receptor Interactions**
**Cellular Regulation of Drug-Receptor Interactions**
**Molecular and Cellular Determinants of Drug Selectivity**
**Drugs That Do Not Fit the Drug-Receptor Model**
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Why is it that one drug affects cardiac function and another alters water and ion balance in the kidney? Why does ciprofloxacin effectively kill bacteria, but rarely harms a patient? These questions can be answered by first examining the interaction between a drug and its specific molecular target and then considering the role of that action in a broader physiologic context. This chapter focuses on the molecular details of drug-receptor interactions, emphasizing the variety of receptors and their molecular mechanisms. This discussion provides a conceptual basis for the action of the many drugs and drug classes discussed in this book. It also serves as a background for Chapter 2, Pharmacodynamics, which discusses the quantitative relationships between drug-receptor interactions and pharmacologic effect.

Although drugs can theoretically bind to almost any three-dimensional target, most drugs achieve their desired (therapeutic) and their toxic effects by interacting with target molecules that play important roles in normal physiologic functioning. In general, **drugs** are molecules that interact with specific molecular components of an organism, and drug **receptors** are macromolecules that, upon binding to a drug, mediate biochemical and physiologic change within the organism.

## Case

Intent on enjoying his newly found retirement, Mr. B has made a point of playing tennis as often as possible during the past year. For the past 3 months, however, he has noted increasing fatigue. Moreover, he was unable to finish a meal, despite being a life-long avid eater. Worried and wondering what these nonspecific symptoms mean, Mr. B schedules an appointment with his doctor. On physical examination, the doctor notes that Mr. B has an enlarged spleen, extending approximately 10 cm below the left costal margin; the physical exam is otherwise within normal limits. Blood tests show an increased total white blood cell count (70,000 cells/mm$^3$) with an absolute increase in neutrophils, band forms, metamyelocytes, and myelocytes, but no blast cells (undifferentiated precursor cells). Cytogenetic analysis of metaphase cells demonstrates that 90% of Mr. B's myeloid cells possess the Philadelphia chromosome (indicating a translocation between chromosomes 9 and 22), confirming the diagnosis of chronic myeloid leukemia. Over the next year, Mr. B receives therapy with interferon, which normalizes his white blood cell count but also gives Mr. B shakes and chills that make him feel as if he is continually sick with the flu. In addition, chromosome analysis reveals that

85% of Mr. B's myeloid cells still possess the Philadelphia chromosome.

Because of the failure to eliminate the Philadelphia chromosome-containing cells and the unpleasant side effects of interferon therapy, the doctor switches Mr. B to imatinib, a specific inhibitor of the BCR-Abl tyrosine kinase that is encoded by the Philadelphia chromosome. Over the next month, the Philadelphia chromosome-containing cells disappear completely from Mr. B's blood, and he begins to feel well enough to compete in a seniors' tennis tournament. Mr. B continues to take imatinib every day, and he has a completely normal blood count and no feelings of fatigue. He is not sure what the future will bring, but he is glad to have been given the chance to enjoy a healthy retirement.

## QUESTIONS

- **1.** How does the BCR-Abl receptor tyrosine kinase alter intracellular signaling pathways?
- **2.** How does imatinib interrupt the activity of the BCR-Abl protein?
- **3.** Why do most standard therapies such as interferon cause significant side effects?
- **4.** Why is imatinib a specific therapy for chronic myeloid leukemia? Why is it that Mr. B does not experience any unpleasant side effects from imatinib therapy?

## CONFORMATION AND CHEMISTRY OF RECEPTORS AND LIGANDS

Why does imatinib act specifically on the BCR-Abl receptor tyrosine kinase and not on other molecules? The answer to this question, and an understanding of why any drug binds to a particular receptor, can be found in the structure of the two molecules and the chemistry this structure imparts on the two actors. This section discusses the basic determinants of receptor structure and the chemistry of drug-receptor binding.

Because many human and microbial drug receptors are proteins, it is useful to review the four major levels of protein structure (Fig. 1-1). At the most basic level, proteins consist of long chains of amino acids, the sequences of which are determined by the sequences of the DNA that code for the proteins. A protein's amino acid sequence is referred to as its **primary structure.** Once a long chain of amino acids has been synthesized on a ribosome, many of the amino acids begin to interact with nearby amino acids in the polypeptide chain. These interactions result in the **secondary structure** of the protein, forming well-defined conformations such as the $\alpha$ helix, $\beta$ pleated sheet, and $\beta$ barrel. As a result of their highly organized shape, these structures often pack tightly with one another, further defining the overall shape of the protein. **Tertiary structure** results from the interaction of amino acids more distal to one another along a single amino acid chain. These interactions include ionic bond formation and the covalent linkage of sulfur atoms to form intramolecular disulfide bridges. Finally, polypeptides may oligomerize to form more complex structures. The conformation that re-

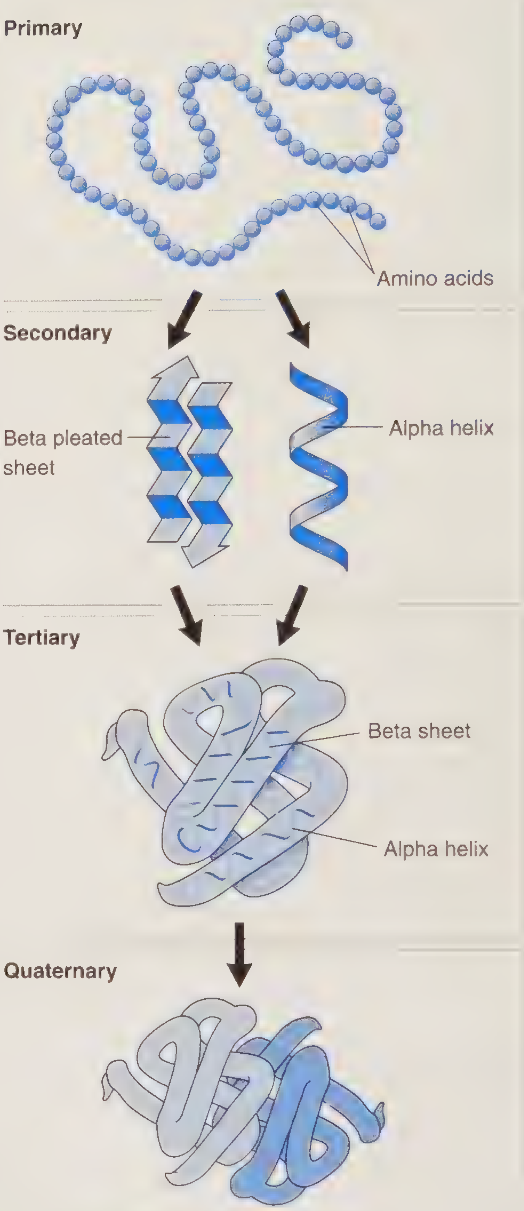


**Figure 1-1. Levels of Protein Structure.** Protein structure can be divided into four levels of complexity, referred to as primary, secondary, tertiary, and quaternary structure. *Primary structure* is determined by the sequence of amino acids that make up the polypeptide chain. *Secondary structure* is determined by the interaction of positively charged hydrogen atoms with negatively charged oxygen atoms on carbons from the same polypeptide chain. These interactions result in a number of characteristic secondary patterns of protein conformation, including the $\alpha$ helix and $\beta$ pleated sheet. *Tertiary structure* is determined by the interactions of amino acids that are relatively far apart on the protein backbone. These interactions give proteins their characteristic three-dimensional structure. *Quaternary structure* is determined by the binding interactions among two or more independent protein subunits.

sults from the interaction of two or more polypeptides is referred to as the **quaternary structure.**

A protein's affinity for water has an additional effect on its shape. Because both the extracellular and the intracellular environments are composed primarily of water, **hydrophobic** protein segments are often drawn to the inside of the protein or shielded from water by insertion into lipid bilayer membranes. Conversely, **hydrophilic** protein segments are often located on a protein's exterior surface. After all of this twisting and turning is completed, each protein has a unique shape that determines its function, location in the body, relationship to cellular membranes, and binding specificity.

Because each of the naturally occurring 20 amino acids has a unique geometry, the three-dimensional orientation of the amino acids that make up a drug-binding site establishes the unique chemical characteristics of the **binding site.** This environment, and the inherent reactivity and shape of the drug, determine the orientation of the drug in relation to the receptor and govern how tightly these molecules bind to one another. Drug-receptor binding is usually the result of multiple chemical interactions between the two molecules, some of which are fairly weak (such as van der Waals forces) and some of which are extremely strong (such as covalent bonding).

**van der Waals forces,** resulting from the induced polarity on a molecule caused by the shifting of its electron density, provide a weak attractive force for some drugs and their receptors. **Hydrogen bonding,** mediated by the interaction between positively polarized atoms (such as hydrogen attached to nitrogen or oxygen) and negatively polarized atoms (such as oxygen, nitrogen, or sulfur), results in bonds of significant strength. Hydrogen bonds give β pleated sheets and α helices their structure. **Ionic interactions,** which occur between atoms with opposite charges, are stronger than hydrogen bonds but less strong than covalent bonds. **Covalent bonding** results from the sharing of a pair of electrons between two atoms on different molecules. The energy required to overcome such interactions is often so great as to make covalent bonding irreversible. Table 1-1 indicates the mechanism of interaction and the relative strength of each of these types of bonds.

*Rarely is drug-receptor binding caused by a single type of interaction; rather, it is a combination of these binding interactions that provides drugs and receptors with the forces necessary to form a stable drug-receptor complex.* In general, the weaker forces comprise the majority of drug-receptor interactions: a typical drug-receptor interaction may consist of 10 or more van der Waals interactions and a few hydrogen bonds; ionic interactions and covalent bonding are much less common. For example, imatinib forms many van der Waals interactions and hydrogen bonds with the ATP-binding site of the BCR-Abl tyrosine kinase. The sum of these forces creates a strong and highly specific interaction between this drug and its receptor (Fig. 1-2).

One additional point about molecular shape is crucial to the specificity of drug-receptor interactions. Most receptor binding pockets are so specific that the **stereochemistry** of the drug has a great impact on the strength of the binding interaction. For example, warfarin is synthesized and administered as a racemic mixture (a mixture containing 50% of the right-handed molecule and 50% of the left-handed molecule), although the S enantiomer is four times more potent than the R because of a stronger interaction of the S form with its binding site on vitamin K epoxide reductase. Stereochemistry can also affect toxicity because one enantiomer of a drug can cause the desired therapeutic effect; whereas the other can cause an undesired toxic effect. The S enantiomer of thalidomide causes defects in fetal development; however, the purified R form of the drug holds promise for becoming a useful treatment for lepromatous leprosy and other conditions. Because it is difficult to produce nonracemic mixtures of organic compounds, manipulating drug stereochemistry provides the pharmaceutical industry with a significant challenge.

## IMPACT OF DRUG BINDING ON RECEPTOR CONFORMATION

The simple act of a drug binding to its receptor is often sufficient to produce an effect. Some drugs act by preventing the binding of endogenous ligands to their receptor binding pockets, without changing the conformation of the receptor. In many drug-receptor interactions, however, the binding of a drug to its receptor results in a change in the conformation of the receptor. Altering the shape of the receptor can affect its function, including enhancing the affinity of the drug for the receptor. Such an interaction is often referred to as **induced fit,** because the receptor's conformation changes so as to improve the quality of the binding interaction.

**TABLE 1-1** Relative Strength of Bonds between Receptors and Drugs

| BOND TYPE | MECHANISM | BOND STRENGTH |
|---|---|---|
| van der Waals | Shifting electron density in areas of a molecule, or in a molecule as a whole, results in the generation of transient positive or negative charges. These areas interact with transient areas of opposite charge on another molecule. | + |
| Hydrogen | Hydrogen atoms bound to nitrogen or oxygen become more positively polarized, allowing them to bond to more negatively polarized atoms such as oxygen, nitrogen, or sulfur. | ++ |
| Ionic | Atoms with an excess of electrons (imparting an overall negative charge on the atom) are attracted to atoms with a deficiency of electrons (imparting an overall positive charge on the atom). | +++ |
| Covalent | Two bonding atoms share electrons. | ++++ |

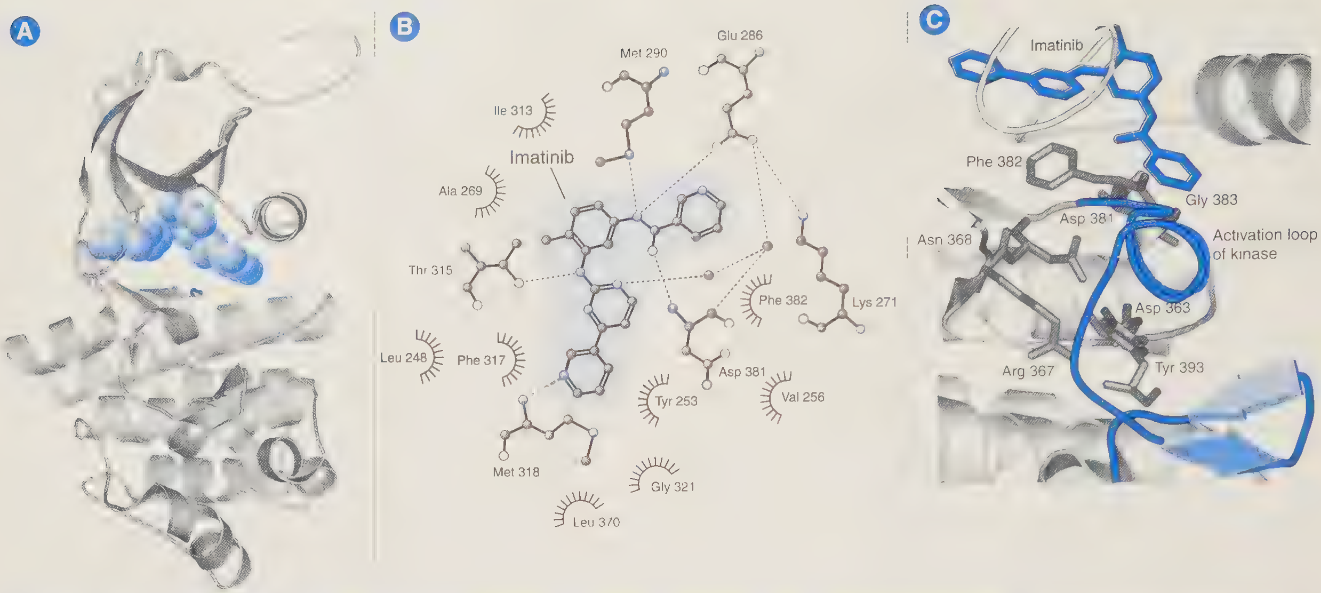


Figure 1-2. Structural Basis of Specific Enzyme Inhibition: Imatinib Interaction with the BCR-Abl Kinase. **A.** The kinase portion of the BCR-Abl tyrosine kinase is shown in a ribbon format (gray). Imatinib, a specific inhibitor of the BCR-Abl tyrosine kinase, is shown as a space-filling model (blue). **B.** Detailed diagram of the intermolecular interactions between imatinib and amino acid residues in the BCR-Abl protein. Hydrogen bonds are indicated by dashed lines, while van der Waals interactions (indicated by halos around the amino acid name and its position in the protein sequence) are shown for nine amino acids with hydrophobic side chains. **C.** The interaction of imatinib (blue) with the BCR-Abl protein (gray) inhibits phosphorylation of a critical activation loop (shown here in a blue-highlighted ribbon format), thus preventing catalytic activity.

The principle of induced fit suggests that drug-receptor binding can have profound effects on the conformation of the receptor. By inducing conformational changes in the receptor, many drugs not only improve the quality of the binding interaction but also alter the action of the receptor. The change in shape induced by the drug is sometimes identical to that caused by the binding of an endogenous ligand. For example, exogenously administered insulin analogues all stimulate the insulin receptor to the same extent, despite their slightly different amino acid sequences. In other cases, drug binding alters the shape of the receptor so as to make it more or less functional than normal. For example, imatinib binding to the BCR-Abl tyrosine kinase causes the protein to assume an inactive conformation, thus inhibiting the kinase activity of the receptor. Quantitative models for these types of interactions are discussed in Chapter 2.

## MEMBRANE EFFECTS ON DRUG-RECEPTOR INTERACTIONS

The structure of the receptor determines not only its binding affinities for drugs and endogenous ligands, but also where the protein lies in relationship to such cellular boundaries as the plasma membrane. Proteins that have large hydrophobic domains are able to reside in the plasma membrane because of the membrane's high lipid content. Many receptors that span the plasma membrane have lipophilic domains that sit in the membrane and hydrophilic domains that reside in the intracellular and extracellular spaces. Other drug receptors, including a number of transcription factors, have only hydrophilic domains and can reside in the cytoplasm, nucleus, or both.

Just as the structure of the receptor determines its location in relationship to the plasma membrane, *the structure of a drug affects its ability to gain access to the receptor*. For example, drugs that are highly water soluble are often less able to pass through the plasma membrane and bind to target molecules in the cytoplasm. In contrast, hydrophilic drugs that are able to pass through transmembrane channels or use other transport mechanisms can gain ready access to cytoplasmic receptors. Drugs that are highly lipophilic, such as many steroid hormones, are able to pass through the hydrophobic lipid environment of the plasma membrane without special channels or transporters and thereby, gain access to intracellular targets.

The ability of drugs to alter receptor shape enables drug binding to receptors on the cell surface to affect functions inside cells. Many cell surface protein receptors have extracellular domains that are linked to intracellular effector molecules through receptor domains that span the plasma membrane. In some cases, changing the shape of the extracellular domain can alter the conformation of the membrane-spanning and/or intracellular domains of the receptor, resulting in a change in receptor function. In other cases, drugs can cross-link the extracellular domains of two receptor molecules, forming a dimeric receptor that activates effector molecules inside the cell.

All of these factors—drug and receptor structure, the chemical forces influencing drug-receptor interaction, drug solubility in water and in the plasma membrane, and the function of the receptor in its cellular environment—confer significant **specificity** on the interactions between drugs and their target receptors. This book discusses numerous examples of drugs that can gain access and bind to receptors in such a way as to induce a conformational change in the receptor, and thereby to produce a biochemical or physiologic effect. This principle suggests that, armed with the knowledge of the structure of a receptor, one could theoretically design a drug that interrupts receptor activity. In fact, much investigation is currently underway to enhance the efficacy and reduce the toxicity of drugs by altering their structure so that they bind more selectively to their targets. This process, referred to as **rational drug design,** has enabled the development of protease inhibitors that have profoundly affected the course of HIV disease as well as antineoplastic agents such as imatinib. This relatively new approach to drug design is discussed in greater detail in Chapter 48, Drug Discovery and Development.

## MAJOR TYPES OF DRUG RECEPTORS

Given the great diversity of drug molecules, it might seem likely that the interactions between drugs and their molecular targets would be equally diverse. This is only partly true. In fact, *most of the currently understood drug-receptor interactions can be classified into four major groups.* These groups comprise the interactions between drugs and (1) transmembrane ion channels, (2) transmembrane receptors coupled to intracellular G proteins, (3) transmembrane receptors with enzymatic cytosolic domains, and (4) intracellular receptors, including enzymes and structural proteins (Fig. 1-3). Table 1-2 provides a summary of each major interaction type.

Knowing whether and to what extent a drug activates or inhibits its target provides valuable information about the interaction. Although **pharmacodynamics** (the effects of drugs on the human body) is covered in detail in the next chapter, it is useful to state briefly the major pharmacodynamic relationships between drugs and their targets before examining the molecular mechanisms of drug-receptor interactions. **Agonists** are molecules that bind to and activate a target. For example, acetylcholine binds to the nicotinic acetylcholine receptor and induces a conformational change in the receptor-associated ion channel from a nonconducting to a conducting state. Not all agonists produce an optimal response upon binding to their targets, however. Such molecules are often called **partial agonists.** Other drugs, known as **antagonists,** inhibit the ability of their targets to be activated by physiologic or pharmacologic agonists. Drugs that directly block the binding site of a physiologic agonist are called **competitive antagonists**; drugs that bind to other sites on the target molecule, and thereby prevent the conformational change required for receptor activation, may be either **noncompetitive** or **uncompetitive antagonists** (see Chapter 2). As the mechanism of each drug-receptor interaction is outlined in the next several sections, it will be useful to consider at a structural level how these different pharmacodynamic effects could be produced.

## TRANSMEMBRANE ION CHANNELS

Many cellular functions require the passage of ions and other hydrophilic molecules across the plasma membrane. Specialized transmembrane channels regulate these processes. The functions of **ion channels** are diverse, including fundamental roles in neurotransmission, cardiac conduction, and muscle contraction. Because of this, drugs targeting ion channels can have a significant impact on major body functions.

Three major mechanisms are used to regulate the activity of transmembrane ion channels. In some channels, the conductance is controlled by ligand binding to the channel. In other channels, the conductance is regulated by changes in voltage across the plasma membrane. In still other channels, the conductance is controlled by ligand binding to plasma membrane receptors that are linked to the channel in some way. The first group of channels is referred to as **ligand-gated,** the second as **voltage-gated,** and the third as **second messenger regulated.** Table 1-3 summarizes the mechanism of activation and function of each channel type.

Channels are generally highly selective for the ions they conduct. For example, action potential propagation in neurons

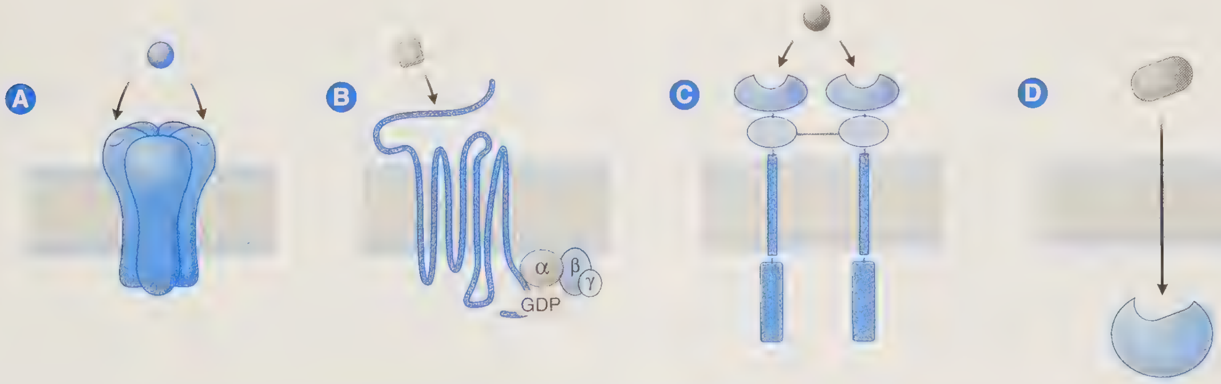


**Figure 1-3. Four Major Types of Interactions Between Drugs and Receptors.** Most drug-receptor interactions can be divided into four groups. **A.** Drugs can bind to ion channels spanning the plasma membrane, causing an alteration in the channel's conductance. **B.** Heptahelical receptors spanning the plasma membrane are functionally coupled to intracellular G proteins. Drugs can influence the actions of these receptors by binding to the extracellular surface or transmembrane region of the receptor. **C.** Drugs can bind to the extracellular domain of a transmembrane receptor and cause a change in signaling within the cell by activating or inhibiting an enzymatic intracellular domain (rectangular box) of the same receptor molecule. **D.** Drugs can diffuse through the plasma membrane and bind to cytoplasmic or nuclear receptors. This is often the pathway used by lipophilic drugs (e.g., drugs that bind to steroid hormone receptors).

**TABLE 1-2** Four Major Types of Drug-Receptor Interactions

| RECEPTOR TYPE | SITE OF DRUG-RECEPTOR INTERACTION | SITE OF RESULTANT ACTION |
|---|---|---|
| Transmembrane ion channel | Extracellular, intrachannel, or intracellular | Cytoplasm |
| Transmembrane linked to intracellular G protein | Extracellular or intramembrane | Cytoplasm |
| Transmembrane with enzymatic cytosolic domain | Extracellular | Cytoplasm |
| Intracellular | Cytoplasm or nucleus | Cytoplasm or nucleus |

of the central and peripheral nervous systems occurs as a result of the synchronous stimulation of voltage-gated ion channels that permit the selective passage of $Na^+$ ions into the cell. When the membrane potential in such a neuron becomes sufficiently positive, the voltage-gated $Na^+$ channels open, allowing a large influx of extracellular sodium ions that further depolarize the cell.

Most ion channels share some structural similarity, regardless of their ion selectivity, the magnitude of their conductance, or their mechanisms of activation (gating) or inactivation. Ion channels tend to be tube-like macromolecules consisting of a number of protein subunits that pass through the plasma membrane. The **ligand-binding domain** can be extracellular, within the channel, or intracellular, while the domain that interacts with other receptors is most often intracellular. The structure of the nicotinic acetylcholine (ACh) receptor has been determined to 4.6 Å resolution, providing an example of the structure of an important ligand-gated ion channel. This receptor consists of five subunits, each of which crosses the plasma membrane (Fig. 1-4). Two of the subunits have been designated α; each contains a single extracellular binding site for ACh. In the free (nonliganded) state of the receptor, the channel is occluded by amino acid side chains and does not allow the passage of ions. Binding of two molecules of acetylcholine to the receptor induces a conformational change that opens the channel and allows ion conductance.

Although the ACh receptor appears to assume only two states, open or closed, many ion channels are able to assume other states as well. For example, some ion channels are able to become **refractory** or **inactivated.** In this state, the channel's permeability cannot be altered for a certain period of time, known as the channel's refractory period. The voltage-gated sodium channel undergoes a cycle of activation, channel opening, channel closing, and channel inactivation. During the inactivation (refractory) period, the channel cannot be reactivated for a number of milliseconds, even if the membrane potential returns to a voltage that normally stimulates the channel to open. Some drugs bind with different affinities to different states of the same ion channel. This **state-dependent binding** is important in the mechanism of action of some local anesthetic and antiarrhythmic drugs, as discussed in Chapters 9 and 17, respectively.

Two important classes of drugs that act by altering the conductance of ion channels are the local anesthetics and the benzodiazepines. Local anesthetics block the conductance of sodium ions through voltage-gated sodium channels in neurons that transmit pain information from the periphery to the central nervous system, thereby preventing action potential propagation and, hence, pain perception (nociception). Benzodiazepines also act on the nervous system, but by a different mechanism. These drugs inhibit neurotransmission in the central nervous system by potentiating the ability of the neurotransmitter GABA to increase the conductance of chloride ions across neuronal membranes, thereby driving the membrane potential further away from its threshold for activation.

## TRANSMEMBRANE G PROTEIN-COUPLED RECEPTORS

**G protein-coupled receptors** are the most abundant class of receptors in the human body. These receptors are exposed at the extracellular surface of the cell membrane, traverse the membrane, and possess intracellular regions that activate a unique class of signaling molecules called G proteins. G protein-coupled signaling mechanisms are involved in many important processes, including vision, olfaction, and neurotransmission.

G protein-coupled receptors all have seven transmembrane regions within a single polypeptide chain. Each transmembrane region consists of a single α helix, and the α helices are arranged in a characteristic structural motif that is similar in all members of this receptor class. The extracellular domain of this class of proteins usually contains the ligand-binding region, although some G protein-coupled receptors bind ligands within the transmembrane domain of the receptor. In the resting (unstimulated) state, the cytoplasmic domain of the

**TABLE 1-3** Three Major Types of Transmembrane Ion Channels

| CHANNEL TYPE | MECHANISM OF ACTIVATION | FUNCTION |
|---|---|---|
| Ligand-gated | Binding of ligand to channel | Altered ion conductance |
| Voltage-gated | Change in transmembrane voltage gradient | Altered ion conductance |
| Second messenger regulated | Binding of ligand to transmembrane receptor with G protein-coupled cytosolic domain, leading to second messenger generation | Second messenger regulates ion conductance of channel |

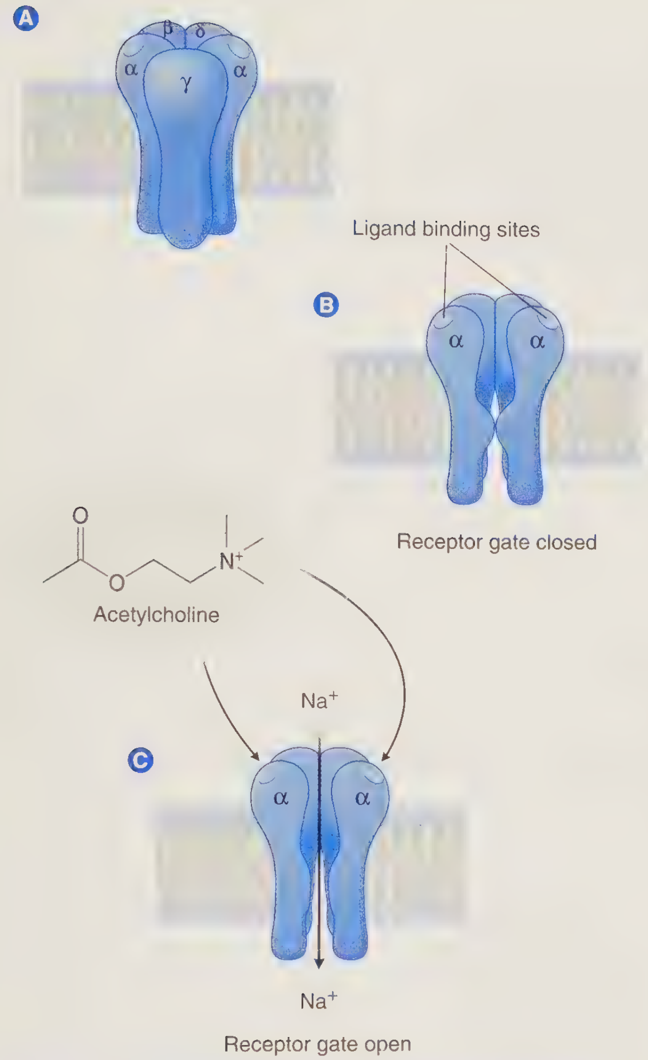


**Figure 1-4. Ligand-Gated Nicotinic Acetylcholine Receptor.** **A.** The plasma membrane acetylcholine (ACh) receptor is composed of five subunits—two $\alpha$ subunits, a $\beta$ subunit, a $\gamma$ subunit, and a $\delta$ subunit. **B.** The $\gamma$ subunit has been removed to show an internal schematic view of the receptor, demonstrating that it forms a transmembrane channel. In the absence of ACh, the receptor gate is closed, and cations [most importantly, sodium ions ($Na^+$)] are unable to traverse the channel. **C.** When ACh is bound to both $\alpha$ subunits, the channel opens, and sodium can pass down its concentration gradient into the cell.

receptor is noncovalently linked to a G protein that consists of $\alpha$ and $\beta\gamma$ subunits. Upon activation, the $\alpha$ subunit exchanges GDP for GTP. The $\alpha$-GTP subunit then dissociates from the $\beta\gamma$ subunit, and the $\alpha$ or $\beta\gamma$ subunit diffuses along the inner leaflet of the plasma membrane to interact with a number of different effectors. These effectors include adenylyl cyclase, phospholipase C, various ion channels, and other classes of proteins. Signals mediated by G proteins are usually terminated by the hydrolysis of GTP to GDP, which is catalyzed by the inherent GTPase activity of the $\alpha$ subunit (Fig. 1-5).

One major role of the G proteins is to activate the production of **second messengers**; that is, signaling molecules that convey the input provided by the first messenger—usually an endogenous ligand or an exogenous drug—to cytoplasmic effectors (Fig. 1-6). The activation of such cyclases as adenylyl cyclase, which catalyzes the production of the second messenger cyclic adenosine-3′,5′-monophosphate (cAMP), and guanylyl cyclase, which catalyzes the production of cyclic guanosine-3′,5′-monophosphate (cGMP), constitutes the most common pathway linked to G proteins. In addition, G proteins can activate the enzyme phospholipase C (PLC) which, among other functions, plays a key role in regulating the concentration of intracellular calcium. Upon activation by a G protein, PLC cleaves the membrane phospholipid phosphatidylinositol-4,5-bisphosphate ($PIP_2$) to the second messengers diacylglycerol (DAG) and inositol-1, 4, 5-trisphosphate ($IP_3$). $IP_3$ triggers the release of $Ca^{2+}$ from intracellular stores, thereby dramatically increasing the intracellular $Ca^{2+}$ concentration and activating downstream molecular and cellular events. DAG activates protein kinase C, which then mediates other molecular and cellular events including smooth muscle contraction and transmembrane ion transport. All of these events are dynamically regulated, so that the different steps in the pathways are activated and inactivated with characteristic kinetics.

A large number of $G\alpha$ protein isoforms have now been identified, each of which has unique effects on its targets. A few of these G proteins include G-stimulatory ($G_s$), G-inhibitory ($G_i$), $G_q$, $G_o$, and $G_{12/13}$. Examples of the effects of these isoforms are shown in Table 1-4. The differential functioning of these G proteins, some of which may couple in different ways to the same receptor in different cell types, is likely to be important for the potential selectivity of future drugs. The $\beta\gamma$ subunits of G proteins can also act as second messenger molecules, although their actions are not as thoroughly characterized.

One important class in the G protein-coupled receptor family is the $\beta$-adrenergic receptor group. The most thoroughly studied of these receptors have been designated $\beta_1$, $\beta_2$, and $\beta_3$. As discussed in more detail in Chapter 8, Adrenergic Pharmacology, $\beta_1$ receptors play a role in controlling heart rate; $\beta_2$ receptors are involved in the relaxation of smooth muscle; and $\beta_3$ receptors play a role in the mobilization of energy by fat cells. Each of these receptors is stimulated by the binding of endogenous catecholamines, such as epinephrine and norepinephrine, to the extracellular domain of the receptor. Epinephrine binding induces a conformational change in the receptor, activating G proteins associated with the cytoplasmic domain of the receptor. The activated (GTP-bound) form of the G protein activates adenylyl cyclase, resulting in increased intracellular cAMP levels and downstream cellular effects. Table 1-5 indicates some of the diverse tissue localizations and actions of the $\beta$-adrenergic receptors.

## TRANSMEMBRANE RECEPTORS WITH ENZYMATIC CYTOSOLIC DOMAINS

The third major class of cellular drug targets consists of transmembrane receptors that transduce an extracellular ligand-binding interaction into an intracellular action through the activation of a linked enzymatic domain. Such receptors play roles in a diverse set of physiologic processes, including cell metabolism, growth, and differentiation. Receptors that have an intracellular enzymatic domain can be grouped into five major classes based on their cytoplasmic mechanism of action (Fig. 1-7). All of these receptors are single–membrane-

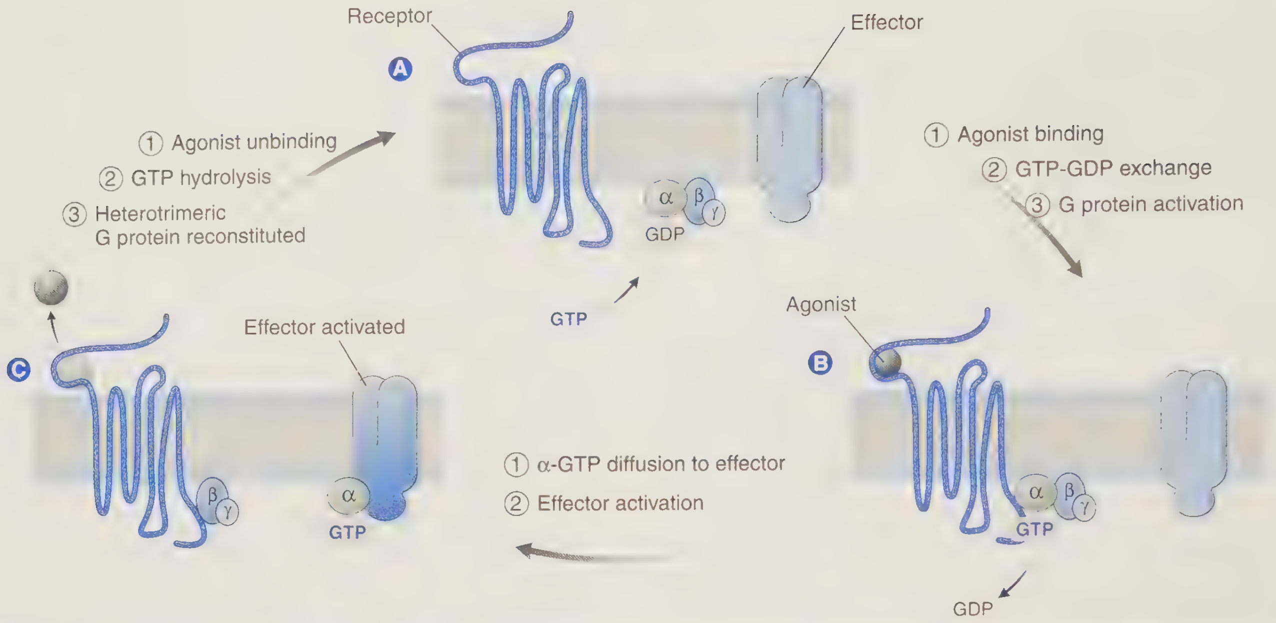


**Figure 1-5. Receptor-Mediated Activation of a G Protein and the Resultant Effector Interaction.** **A.** In the resting state, the α and βγ subunits of a G protein are associated with one another, and GDP is bound to the α subunit. **B.** Binding of an extracellular ligand (agonist) to a G protein-coupled receptor causes the exchange of GTP for GDP on the α subunit. **C.** The βγ subunit dissociates from the α subunit, which diffuses to interact with effector proteins. Interaction of the GTP-associated α subunit with an effector activates the effector. In some cases (not shown), the βγ subunit can also activate effector proteins. Depending on the receptor subtype and the specific Gα isoform, Gα can also inhibit the activity of an effector molecule. The α subunit possesses intrinsic GTPase activity, which leads to hydrolysis of GTP to GDP. This leads to reassociation of the α subunit with the βγ subunit and the cycle can begin again.

spanning proteins, in contrast to the seven-membrane-spanning motif present in G protein-coupled receptors. Another important mechanistic distinction is that many receptors with enzymatic cytosolic domains form dimers or multisubunit complexes to transduce their signals.

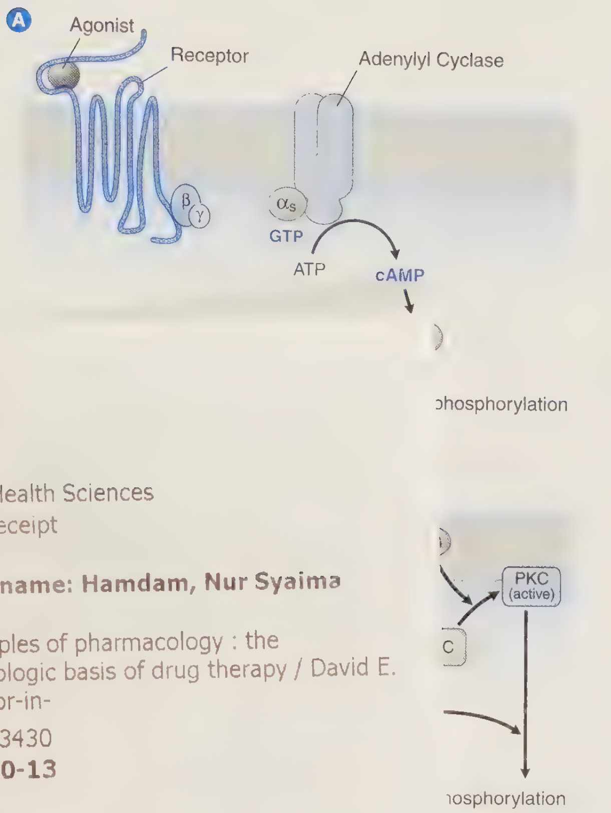
Many of the receptors with enzymatic cytosolic domains modify proteins by adding or removing phosphate groups to or from specific amino acid residues. *Phosphorylation is a ubiquitous mechanism of protein signaling.* The large negative charge of phosphate groups can dramatically alter the three-dimensional structure of a protein and, thus, change that protein's activity. In addition, phosphorylation is easily reversible, thus allowing this signaling mechanism to act specifically in time and space.

## Receptor Tyrosine Kinases

The largest group of transmembrane receptors with enzymatic cytosolic domains is the receptor tyrosine kinase family. These receptors transduce the signals from many hormones and growth factors by phosphorylating tyrosine residues on the cytoplasmic tail of the receptor. This leads to recruitment and subsequent tyrosine phosphorylation of a number of cytosolic signaling molecules.

The insulin receptor is a well-characterized receptor tyrosine kinase. This receptor consists of two extracellular α subunits that are covalently linked to two membrane-spanning β subunits. Binding of insulin to the α subunits results in a change in conformation of the adjacent β subunits, causing the β subunits to move closer to one another on the intracellular face of the membrane. The proximity of the two β subunits promotes a transphosphorylation reaction, in which one β subunit phosphorylates the other ("autophosphorylation"). The phosphorylated tyrosine residues then act to recruit other cytosolic proteins, known as insulin receptor substrate (IRS) proteins. Type 2 diabetes mellitus may in some cases be associated with defects in postinsulin receptor signaling; thus, understanding the insulin receptor signaling pathways is relevant for the potential design of rational therapeutics. The mechanism of insulin receptor signaling is discussed in further detail in Chapter 28, Pharmacology of the Endocrine Pancreas.

Recognizing that receptor tyrosine kinases play an important role in cellular growth and differentiation, it is not surprising that "gain-of-function" mutations in these receptors (i.e., mutations that cause ligand-independent activity of these molecules) can lead to uncontrolled cell growth and cancer. Recall from the introductory case that chronic myeloid leukemia is associated with the Philadelphia chromosome, which results from a reciprocal translocation between the long arms of chromosomes 9 and 22. The mutant chromosome codes for a constitutively active receptor tyrosine kinase referred to as the BCR-Abl protein (BCR and Abl are short for "break-point cluster region" and "Abelson," respectively, the two chromosomal regions that undergo translocation with high frequency in this form of leukemia). Continued activity of this kinase results in phosphorylation of a number of cytosolic proteins, leading to dysregulated myeloid cell growth and chronic myeloid leukemia. Imatinib inhibits BCR-Abl activity by neutralizing its ability to phosphorylate substrates. This is the first example of a drug targeted specifically to receptor tyrosine kinases, and its success will likely lead to the development of a number of drugs that act by similar mechanisms.



rookfield Health Sciences
orrower Receipt

**ustomer name: Hamdam, Nur Syaima**

itle: Principles of pharmacology : the
athophysiologic basis of drug therapy / David E.
olan, editor-in-

D: 0009133430
**ue: 04-10-13**

otal items: 1
7/09/2013 14:31
hecked out: 3

Thank you for using the self service system.
Please keep this receipt for the due date.
SC5

**and Phospholi-**
act with several
e of Gα protein
the G protein
its are $G\alpha_s$ and
holipase C, re-
yclase converts
rotein kinase A
cytosolic pro-
C (PLC) cleaves
l-4,5-bisphos-
l-1,4,5-trisphos-
vate protein
c cellular pro-
plasmic reticu-
lum into the cytosol. Calcium release also stimulates protein phosphorylation events that lead to changes in protein activation. Although not shown, the βγ subunits of G proteins can also affect certain cellular signal transduction cascades.

**TABLE 1-4** The Major G Proteins and Examples of Their Actions

| G PROTEIN | ACTIONS |
|---|---|
| G-stimulatory ($G_s$) | Activates $Ca^{2+}$ channels, activates adenylyl cyclase |
| G-inhibitory ($G_i$) | Activates $K^+$ channels, inhibits adenylyl cyclase |
| $G_o$ | Inhibits $Ca^{2+}$ channels |
| $G_q$ | Activates phospholipase C |
| $G_{12/13}$ | Diverse ion transporter interactions |

## Receptor Tyrosine Phosphatases

Just as receptor tyrosine kinases phosphorylate the tyrosine residues of cytoplasmic proteins, receptor tyrosine phosphatases remove phosphate groups from specific tyrosine residues. In some cases, this may be an example of receptor convergence (discussed later), where the differential effects of two receptor types can negate one another. However, receptor tyrosine phosphatases possess novel signaling mechanisms as well. Many receptor tyrosine phosphatases are found in immune cells, where they regulate cell activation. These receptors are discussed further in Chapter 43, Pharmacology of Immunosuppression.

## Tyrosine Kinase-Associated Receptors

Tyrosine kinase-associated receptors constitute a diverse family of proteins that, although lacking inherent catalytic activity, recruit active cytosolic signaling proteins. These are also called (somewhat confusingly) nonreceptor tyrosine kinases. Ligand activation of cell surface tyrosine kinase-associated receptors causes the receptors to cluster together. This clustering event recruits cytoplasmic proteins that are then activated to phosphorylate other proteins on tyrosine residues. Thus, the downstream effect is much like that of receptor tyrosine kinases, except that tyrosine kinase-associated receptors rely on a nonreceptor kinase to phosphorylate target proteins. Important examples of tyrosine kinase-associated receptors include cytokine receptors and a number of other receptors in the immune system. These receptors are discussed in detail in Chapter 43.

**TABLE 1-5** β-Adrenergic Receptors: Tissue Localization and Action

| RECEPTOR | TISSUE LOCALIZATION | ACTION |
|---|---|---|
| $\beta_1$ | SA node of heart | Increases heart rate |
| | Cardiac muscle | Increases contractility |
| | Adipose tissue | Increases lipolysis |
| $\beta_2$ | Bronchial smooth muscle | Dilates bronchioles |
| | Gastrointestinal smooth muscle | Constricts sphincters and relaxes gut wall |
| | Uterus | Relaxes uterine wall |
| | Bladder | Relaxes bladder |
| | Liver | Increases gluconeogenesis and glycolysis |
| | Pancreas | Increases insulin release |
| $\beta_3$ | Adipose tissue | Increases lipolysis |

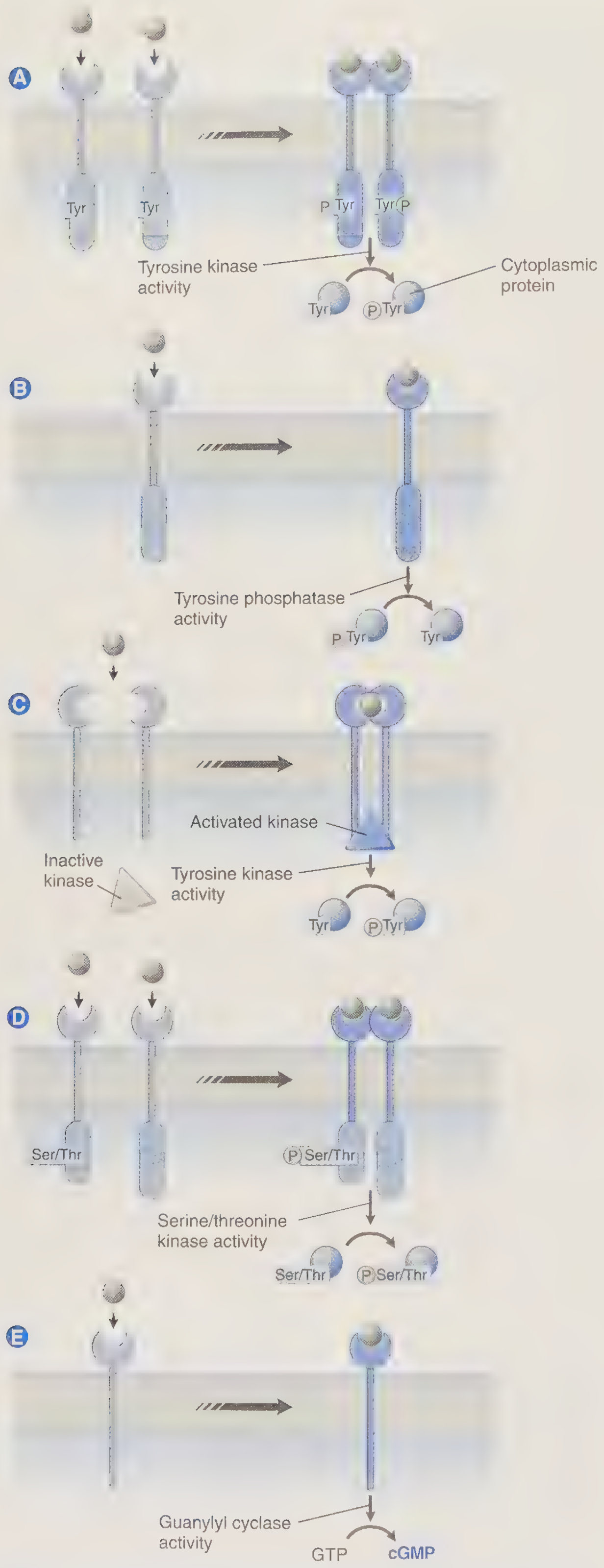


**Figure 1-7. Major Types of Transmembrane Receptors with Enzymatic Cytosolic Domains.** There are five major categories of transmembrane receptors with enzymatic cytosolic domains. **A.** The largest group is comprised of receptor tyrosine kinases. After ligand-induced activation, these receptors dimerize and transphosphorylate tyrosine residues in the receptor and, often, on target cytosolic proteins. Examples of receptor tyrosine kinases include the insulin receptor and the BCR-Abl protein. **B.** Some receptors can act as tyrosine phosphatases. These receptors dephosphorylate tyrosine residues either on other transmembrane receptors or on cytosolic proteins. Many cells of the immune system have receptor tyrosine phosphatases. **C.** Some tyrosine kinase-associated receptors lack a definitive enzymatic domain, but binding of ligand to the receptor triggers activation of receptor-associated protein kinases (termed nonreceptor tyrosine kinases) that then phosphorylate tyrosine residues on certain cytosolic proteins. **D.** Receptor serine/threonine kinases phosphorylate serine and threonine residues on certain target cytosolic proteins. Members of the TGF-β superfamily of receptors are in this category. **E.** Receptor guanylyl cyclases contain a cytosolic domain that catalyzes the formation of cGMP from GTP. The receptor for B-type natriuretic peptide is one of the receptor guanylyl cyclases that has been well characterized.

## Receptor Serine/Threonine Kinases

These receptors, which are all members of the transforming growth factor β (TGF-β) receptor superfamily, are important mediators of cell growth and differentiation that have been implicated in cancer progression and metastasis. They act by phosphorylating serine and threonine residues on cytoplasmic target proteins. No pharmacologic agents currently exist that target serine/threonine kinases, although such drugs are under development.

## Receptor Guanylyl Cyclases

Recall that the activation of G protein-coupled receptors may cause Gα subunits to alter the activity of adenylyl and guanylyl cyclases. Receptor guanylyl cyclases have no intermediate G protein. Instead, ligand binding stimulates intrinsic receptor guanylyl cyclase activity, in which GTP is converted to cGMP. This is the smallest family of transmembrane receptors. B-type natriuretic peptide, a hormone secreted by the ventricles in response to volume overload, acts via a receptor guanylyl cyclase. A recombinant version of this peptide has recently been approved for treatment of decompensated heart failure, as discussed in Chapter 19, Pharmacology of Volume Regulation.

# INTRACELLULAR RECEPTORS

The transcription regulatory factors are important cytosolic receptors that are targeted by lipophilic drugs. All proteins in the body are encoded by DNA. The transcription of DNA into RNA and the translation of RNA into protein are controlled by a diverse set of molecules. Transcription of many genes is regulated, in part, by the interaction between lipid-soluble signaling molecules and transcription regulatory factors. Because of the fundamental role played by control of transcription in many biological processes, **transcription factors** are the targets of some drugs. **Steroid hormones** are an important class of lipophilic drugs that can diffuse readily through the plasma membrane and achieve their actions by binding to transcription factors in the cytoplasm or nucleus (Fig. 1-8).

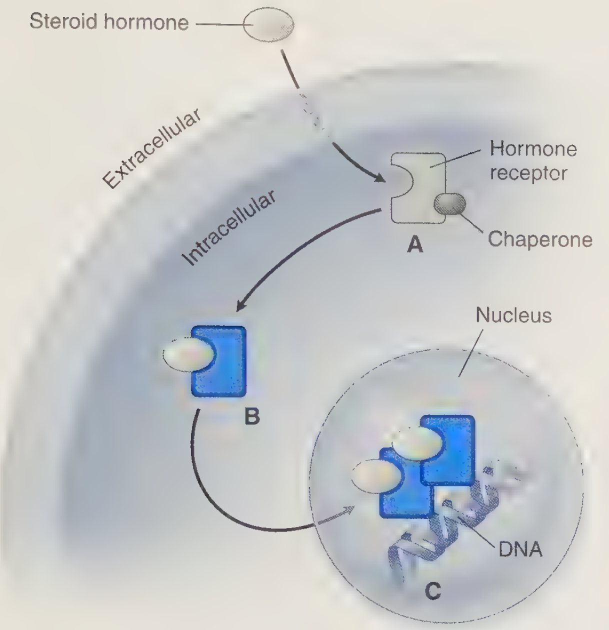


**Figure 1-8. Lipophilic Molecule Binding to an Intracellular Transcription Factor.** **A.** Small lipophilic molecules can diffuse through the plasma membrane and bind to intracellular transcription factors. In this example, steroid hormone binding to a cytosolic hormone receptor is shown, although some receptors of this class may be located in the nucleus before ligand binding. **B.** Ligand binding triggers a conformational change in the receptor (and often, as shown here, dissociation of a chaperone repressor protein) that leads to transport of the ligand-receptor complex into the nucleus. In the nucleus, the ligand-receptor complex typically dimerizes. In the example shown, the active form of the receptor is a homodimer (two identical receptors binding to one another), but heterodimers (such as the thyroid hormone receptor and the retinoid X receptor) may also form. **C.** The dimerized ligand-receptor complex binds to DNA, and may then recruit coactivators or corepressors (not shown). These complexes alter the rate of gene transcription, leading to a change (either up or down) in cellular protein expression.

Just as the shape of a transcription factor governs to which drugs it will bind, the shape also determines where on the genome the transcription factor will attach. By activating or inhibiting transcription, thereby altering the intracellular or extracellular concentrations of specific gene products, drugs that target transcription factors can have a great impact on cellular function. The cellular responses to such drugs, and the effects that result from this cellular response in tissues and organ systems, provide links between the molecular drug-receptor interaction and the effects of the drug on the organism as a whole. Because gene transcription is a relatively slow (minutes to hours) and long-lasting process, drugs that target transcription factors often require a longer period of time for the onset of action to take place, and have longer-lasting effects, than do drugs that alter more transient processes, such as ion conduction (seconds to minutes).

Not all drugs with cytosolic targets act on transcription factors. **Structural proteins,** such as tubulin, are important targets for antineoplastic drugs that are able to diffuse through the plasma membrane of the cancer cell. For example, the antimitotic vinca alkaloids bind to tubulin monomers and prevent the polymerization of this molecule into microtubules. This inhibition of microtubule formation arrests the affected cells in metaphase. Other drugs bind directly to RNA or ribosomes; such drugs are important agents in antimicrobial and antineoplastic chemotherapy. **Enzymes** are also a common cytosolic target. Vitamin K epoxide reductase, a cytosolic enzyme involved in the post-translational addition of glutamate residues to clotting factors, is the target of the anticlotting agent warfarin. Finally, many lipophilic inhibitors of cytosolic **signal transduction molecules** are under development, including drugs that target mediators of apoptosis (programmed cell death) or inflammation.

## PROCESSING OF SIGNALS RESULTING FROM DRUG-RECEPTOR INTERACTIONS

Many cells in the body are continuously bombarded with multiple inputs, some stimulatory and some inhibitory. How do cells integrate these signals to produce a coherent effect? G proteins and other second messengers seem to provide important points of integration. As noted above, relatively few second messengers have been identified, and it is unlikely that many more remain to be discovered. Thus, second messengers are an attractive candidate mechanism for providing cells with a set of common points upon which numerous outside stimuli could converge to generate a coordinated cellular effect (Fig. 1-9).

Ion concentrations provide another point of integration for cellular effects because the cellular concentration of a particular ion is the result of the integrated activity of *multiple* ionic currents that both enhance and diminish the concentration of the ion within the cell. For example, the contractile state of a smooth muscle cell is a function of the intracellular calcium ion concentration, which is determined by several different $Ca^{2+}$ conductances. These conductances include calcium ion leaks into the cell and calcium currents into and out of the cytoplasm through specialized channels in the plasma membrane and smooth endoplasmic reticulum.

Because the magnitude of cellular response is often considerably greater than the magnitude of the stimulus that caused the response, cells seem to have the ability to amplify the effects of receptor binding. G proteins provide an excellent example of signal amplification. Ligand binding to a G protein-coupled receptor serves to activate a single G protein molecule. This G protein molecule can then bind to and activate many effector molecules, such as adenylyl cyclase, which can then generate an even greater number of second messenger molecules. Another example of signal amplification is "trigger $Ca^{2+}$," in which a small influx of $Ca^{2+}$ through voltage-gated $Ca^{2+}$ channels in the plasma membrane "triggers" the release of larger amounts of $Ca^{2+}$ into the cytoplasm from intracellular stores.

## CELLULAR REGULATION OF DRUG-RECEPTOR INTERACTIONS

Drug-induced activation or inhibition of a receptor often has a lasting impact on the receptor's subsequent responsiveness to drug binding. Mechanisms that mediate such effects are

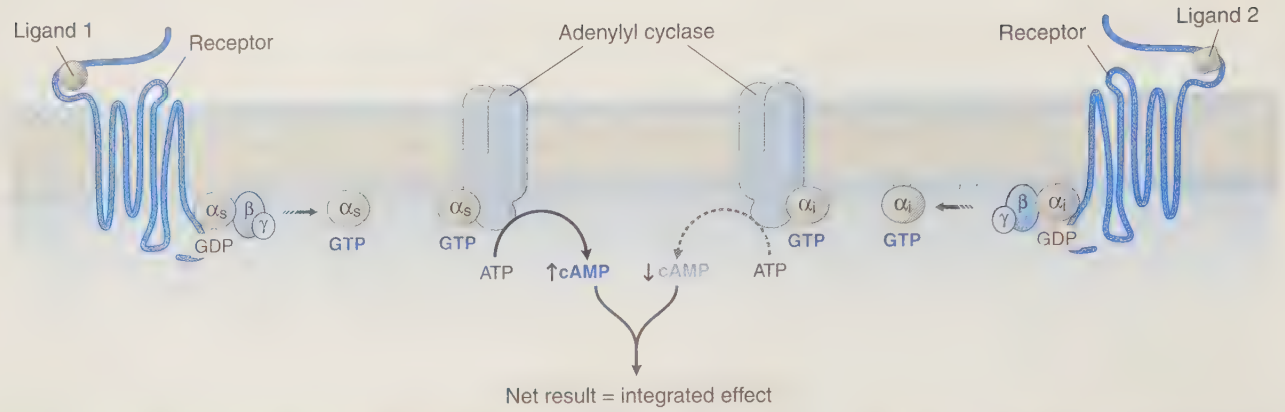


**Figure 1-9. Signaling Convergence of Two Receptors.** A limited number of mechanisms are used to transduce intracellular signal cascades. In some cases, this allows for convergence, where two different receptors have opposite effects that tend to negate one another in the cell. In a simple example, two different G protein-coupled receptors could be stimulated by different ligands. The receptor shown on the left is coupled to $G\alpha_s$, a G protein that stimulates adenylyl cyclase to catalyze the formation of cAMP. The receptor shown on the right is coupled to $G\alpha_i$, a G protein that inhibits adenylyl cyclase. When both of these receptors are activated simultaneously, they can attenuate or even neutralize each other, as shown. Sometimes, signaling through a pathway may alternate as the two receptors are sequentially activated.

important because they prevent overstimulation that could lead to cellular damage or adversely affect the organism as a whole. Many drugs show diminishing effects over time; this phenomenon is called **tachyphylaxis.** In pharmacologic terms, the receptor and the cell become **desensitized** to the action of the drug. Mechanisms of desensitization can be divided into two types: **homologous,** in which the actions of agonists at only one class of receptors are lessened; and **heterologous,** in which the effects of agonists on a number of classes of receptors are coordinately diminished. Heterologous desensitization is thought to be caused by drug-induced alteration in a common point of convergence in the mechanisms of action of the involved receptors, such as a shared effector molecule.

Many receptors exhibit desensitization. For example, the cellular response to repeated stimulation of β-adrenergic receptors by epinephrine diminishes steadily over time (Fig. 1-10). β-adrenergic receptor desensitization is mediated by epinephrine-induced phosphorylation of the cytoplasmic tail of the receptor. This phosphorylation promotes the binding of β-arrestin to the receptor; in turn, β-arrestin inhibits the receptor's ability to stimulate the G protein $G_s$. With lower levels of activated $G_s$ present, adenylyl cyclase produces less cAMP. In this manner, repeated cycles of ligand-receptor binding result in smaller and smaller cellular effects. Other molecular mechanisms have even more profound effects, completely turning off the receptor to stimulation by ligand. The latter phenomenon, referred to as **inactivation,** may also result from phosphorylation of the receptor; in this case, the phosphorylation completely blocks the signaling activity of the receptor.

Another mechanism that can affect the cellular response caused by drug-receptor binding is called **refractoriness.** Receptors that assume a refractory state following activation require a period of time to pass before they can be stimulated again. As noted above, voltage-gated sodium channels, which mediate the firing of neuronal action potentials, are subject to refractory periods. Following channel opening induced by membrane depolarization, the voltage-gated sodium channel spontaneously closes, and cannot be reopened for some period of time (called the **refractory period**). This inherent property of the channel determines the maximum rate at which neurons can be stimulated and transmit information.

The effect of drug-receptor binding can also be influenced by drug-induced changes in the number of receptors on or in a cell. One example of a molecular mechanism by which receptor number can be altered is called **down-regulation.** In this phenomenon, prolonged receptor stimulation by ligand induces the cell to endocytose and sequester receptors in endocytic vesicles. This sequestration prevents the receptors from coming into contact with ligands, resulting in cellular desensitization. When the stimulus that caused the receptor sequestration subsides, the receptors can be recycled to the cell surface and, thereby, rendered functional again (Fig. 1-10). Cells also have the ability to alter the level of synthesis of receptors and, thereby, to regulate the number of receptors available for drug binding. Receptor sequestration and alteration in receptor synthesis occur on a longer time scale than does phosphorylation, and have longer-lasting effects as well. Table 1-6 provides a summary of the mechanisms by which the effects of drug-receptor interactions can be regulated.

## MOLECULAR AND CELLULAR DETERMINANTS OF DRUG SELECTIVITY

The ideal drug would interact only with a molecular target that causes the desired therapeutic effect but not with molecular targets that cause unwanted toxic side effects. Although no such drug has yet been discovered (i.e., all drugs currently in clinical use have the potential to cause toxic side effects as well as therapeutic effects), pharmacologists can take advantage of several determinants of drug **selectivity** in an attempt to reach this goal. Selectivity of drug action can be conferred by at least two classes of mechanisms, including (1) the cell-

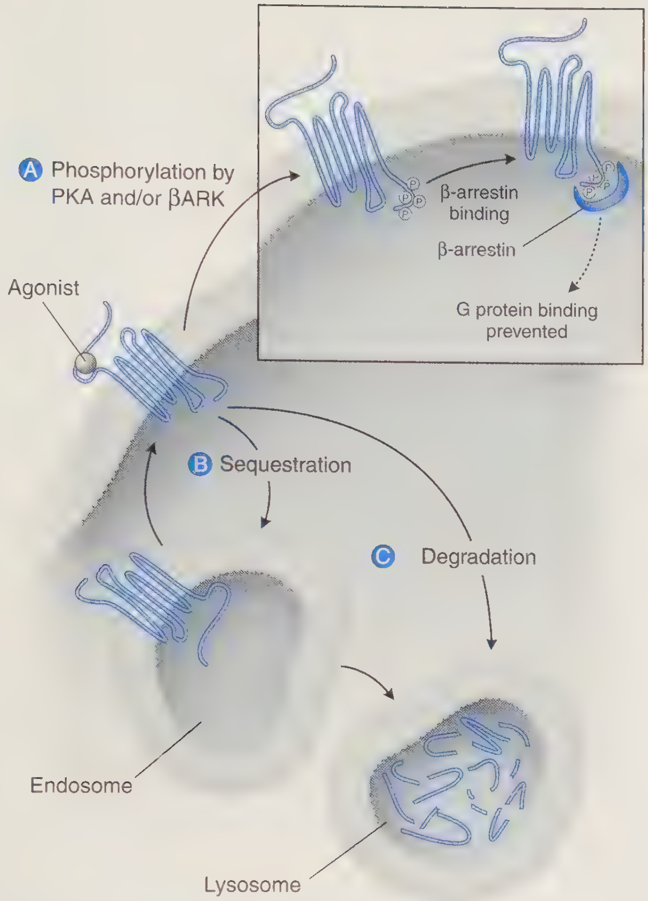


**Figure 1-10. β-Adrenergic Receptor Regulation.** Agonist-bound β-adrenergic receptors activate G proteins, which then stimulate adenylyl cyclase activity. **A.** Repeated or persistent stimulation of the receptor by agonist results in phosphorylation of amino acids at the C-terminus of the receptor by protein kinase A (PKA) and/or β-adrenergic receptor kinase (βARK). β-Arrestin then binds to the phosphorylated domain of the receptor and blocks $G_s$ binding, thereby decreasing adenylyl cyclase (effector) activity. **B.** Binding of β-arrestin also leads to receptor sequestration into endosomal compartments, effectively neutralizing β-adrenergic receptor signaling activity. The receptor can then be recycled and reinserted into the plasma membrane. **C.** Prolonged receptor occupation by agonist can lead to receptor down-regulation and eventual receptor degradation. Cells can also reduce the number of surface receptors by inhibiting the transcription or translation of the gene coding for the receptor (not shown).

type specificity of receptor subtypes, and (2) the cell-type specificity of receptor-effector coupling.

Although many potential receptors for drugs are widely distributed among diverse cell types, some receptors are more limited in their distribution. Systemic administration of drugs that interact with such receptors can result in a highly selective therapeutic effect. For example, drugs that target such ubiquitous processes as DNA synthesis are likely to cause significant toxic side effects; this is the case with many currently available chemotherapeutics for the treatment of cancer. Other drugs that target such cell-type restricted processes as acid generation in the stomach may have fewer toxic side effects. Imatinib is an extremely selective drug because the BCR-Abl protein is not expressed in normal (noncancerous) cells. In general, *the more restricted the cell-type distribution of the receptor targeted by a particular drug, the more selective the drug is likely to be.*

Similarly, even though many different cell types may express the same molecular target for a drug, the effect of that drug may differ in the various cell types because of differential receptor–effector coupling mechanisms or differential requirements for the drug target in the various cell types. For example, although voltage-gated calcium channels are ubiquitously expressed in the heart, cardiac pacemaker cells are relatively more sensitive to the effects of calcium channel blocking agents than are cardiac ventricular muscle cells. This differential effect is attributable to the fact that action potential propagation depends mainly on the action of calcium channels in cardiac pacemaker cells; whereas, sodium channels are more important than calcium channels in the action potentials of ventricular muscle cells. In general, *the more the receptor–effector coupling mechanisms differ among the various cell types that express a particular molecular target for a drug, the more selective the drug is likely to be.*

## DRUGS THAT DO NOT FIT THE DRUG-RECEPTOR MODEL

Although many drugs interact with the four basic receptor types outlined above, others act by nonreceptor-mediated mechanisms. Two examples are the osmotic diuretics and the antacids.

**TABLE 1-6** Mechanisms of Receptor Regulation

| MECHANISM | DEFINITION |
|---|---|
| Tachyphylaxis | Repeated administration of the same dose of a drug results in a reduced effect of the drug over time |
| Desensitization | Decreased ability of a receptor to respond to stimulation by a drug or ligand |
| 1. Homologous | 1. Decreased response of a single class of receptor |
| 2. Heterologous | 2. Decreased response of multiple classes of receptor |
| Inactivation | Loss of ability of a receptor to respond to stimulation by a drug or ligand |
| Refractory | After a receptor is stimulated, a period of time is required before the next drug-receptor interaction can produce an effect |
| Down-regulation | Repeated or persistent drug-receptor interaction results in removal of the receptor from sites where subsequent drug-receptor interactions could take place |

Diuretics control fluid balance in the body by altering the relative levels of water and ion absorption and secretion in the kidney. Many of these drugs act on ion channels. One class of diuretics, however, alters water and ion balance not by binding to ion channels or G protein-coupled receptors, but by changing the osmolarity in the nephron directly. The sugar mannitol is secreted into the lumen of the nephron and increases the osmolarity of the urine to such a degree that water is drawn from the peritubular blood into the lumen. This fluid shift serves to increase the volume of urine while decreasing the blood volume.

Another class of drugs that does not fit the drug-receptor model is the antacids, which are used to treat gastroesophageal reflux disease and peptic ulcer disease. Unlike antiulcer agents that bind to receptors involved in the physiologic generation of gastric acid, antacids act nonspecifically by absorbing or chemically neutralizing stomach acid. Examples of these agents include bases such as $NaHCO_3$ and $Mg(OH)_2$.

## Conclusion and Future Directions

Although the molecular details of drug-receptor interactions vary widely among drugs of different classes and receptors of different types, the four fundamental mechanisms of action described in this chapter serve as paradigms of the principles of pharmacodynamics. The ability to classify drugs based on their mechanisms of action makes it possible to simplify the study of pharmacology, because the molecular mechanism of action of a drug can usually be linked to its cellular, tissue, organ, and system levels of action. It is then possible to understand how a given drug mediates its particular therapeutic effects and its unwanted or toxic side effects in a particular patient.

## Suggested Readings

Pratt WB, Taylor P, eds. Principles of Drug Action: The Basis of Pharmacology, 3rd Ed. New York: Churchill Livingstone, 1990. *(Contains a detailed discussion of drug-receptor interactions.)*

Berg JM, Tymoczko JL, Stryer L. Biochemistry, 5th Ed. New York: WH Freeman and Company, 2002. *(Contains structural data on receptors, especially G proteins.)*

Neves SR, Ram PT, Iyengar R. G protein pathways. Science 2002; 296:1636–1639. *(Current understanding of the many roles G proteins play in cellular signaling.)*

Savage DG, Antman KH. Imatinib mesylate—A new targeted therapy. N Engl J Med 2002;346:683–693. *(Summarizes the pathophysiologic basis for chronic myeloid leukemia and reviews the mechanistic basis for imatinib specificity.)*

# 2

# Pharmacodynamics

Harris S. Rose and David E. Golan

**Introduction**
**Case**
**Drug-Receptor Binding**
**Dose-Response Relationships**
  Graded Dose–Response Relationships
  Quantal Dose–Response Relationships
**Drug-Receptor Interactions**
  Agonists
  Antagonists
    *Competitive Receptor Antagonists*
    *Noncompetitive Receptor Antagonists*
    *Nonreceptor Antagonists*
  Partial Agonists
  Inverse Agonists
  Spare Receptors
**Concepts in Therapeutics**
  Therapeutic Index and Therapeutic Window
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

*Pharmacodynamics is the term used to describe quantitatively the effects of a drug on the body.* The previous chapter described the molecular interactions by which pharmacologic agents exert their effects. The integration of these molecular actions into an effect on the organism as a whole is the subject addressed in this chapter. It is important to describe the effects of a drug quantitatively in order to determine appropriate dose ranges for patients, as well as to compare the potency and efficacy of one drug to that of another.

## Case

Admiral X is a 66-year-old retired submarine captain with a 70 pack-year smoking history (two packs per day for 35 years) and a family history of coronary artery disease. Although he usually ignores the advice of his physicians, he does take his prescribed pravastatin to reduce his cholesterol level and aspirin to reduce his risk of coronary artery occlusion.

One day, while working in his woodshop, Adm. X begins to feel tightness in his chest. The feeling rapidly becomes painful, and the pain begins to radiate down his left arm. He calls 911, and an ambulance transports him to the local emergency room. After evaluation, it is determined that Adm. X is having an anterior myocardial infarction. Because he has no specific contraindications to thrombolytic therapy (such as uncontrolled hypertension, history of stroke, or recent surgery), the physician initiates therapy with both a thrombolytic agent, tissue-type plasminogen activator (tPA), and an anticoagulant, heparin. Improper dosing of both of these drugs can have dire consequences (hemorrhage and death) because of their low therapeutic indices, so Admiral X is closely monitored, and the pharmacologic effect of the heparin is measured periodically by testing the partial thromboplastin time (PTT). Adm. X's symptoms resolve over the next several hours, although he remains in the hospital for monitoring. He is discharged after 4 days in the hospital; his discharge medications include pravastatin, aspirin, atenolol, lisinopril, and clopidogrel for secondary prevention of myocardial infarction.

## QUESTIONS

1. How does the molecular interaction of a drug with its receptor determine the potency and efficacy of the drug?
2. What properties of certain drugs, such as aspirin, allow them to be taken without monitoring of plasma drug levels; whereas, other drugs, such as heparin, require such monitoring?
3. Why does the fact that a drug has a low therapeutic index mean that the physician must use greater care in its administration?

## DRUG-RECEPTOR BINDING

The study of pharmacodynamics is based on the concept of drug-receptor binding. When either a drug or an endogenous ligand (such as a hormone or neurotransmitter) binds to its receptor, a response may result from that binding interaction. When a sufficient number of receptors are bound (or "occupied") on or in a cell, the cumulative effect of receptor "occupancy" may become apparent in that cell. At some point, all of the receptors may be occupied, and a maximal response may be observed. When the response occurs in many cells, the effect can be seen at the level of the organ or even the patient. But this all starts with the binding of drug or ligand to a receptor (for the purpose of discussion, "drug" and "ligand" will be used interchangeably for the remainder of this chapter). A model that accurately describes the binding of drug to receptor would, therefore, be useful in predicting the effect of the drug at the molecular, cellular, tissue (organ), and organism (patient) levels. This section describes one such model.

Consider the simplest case, in which receptor is either free (unoccupied) or reversibly bound to drug (occupied). We can describe this case as follows:

$$L + R \overset{K_d}{\rightleftarrows} LR \qquad \text{Equation 2-1}$$

where $L$ is ligand (drug), $R$ is free receptor, and $LR$ is bound drug-receptor complex. At equilibrium, the fraction of receptors in each state is dependent upon the dissociation constant, $K_d$. $K_d$ is an intrinsic property of any given drug-receptor pair. Although $K_d$ varies with temperature, the temperature of the human body is relatively constant, and it can therefore be assumed that $K_d$ is a constant for each drug-receptor combination.

According to the law of mass action, the relationship between free and bound receptor can be described as follows:

$$K_d = \frac{[L][R]}{[LR]}, \text{ rearranged to } [LR] = \frac{[L][R]}{K_d} \qquad \text{Equation 2-2}$$

where $[L]$ is free ligand concentration, $[R]$ is free receptor concentration, and $[LR]$ is ligand–receptor complex concentration. Because $K_d$ is a constant, some important properties of the drug-receptor interaction can be deduced from this equation. First, as ligand concentration is increased, the concentration of bound receptors increases. Second, and not so obvious, is that as free receptor concentration is increased (as it may, for example, in disease states or upon repeated exposure to a drug), bound receptor concentration also increases. Therefore, *an increase in the effect of a drug can result from an increase in the concentration of either the ligand or the receptor.* The remainder of the discussion in this chapter, however, assumes that the total concentration of receptors is a constant, so that $[LR]+[R]=[R_o]$. This allows Equation 2-2 to be arranged as follows:

$$[R_o] = [R] + [LR] = [R] + \frac{[L][R]}{K_d}$$

$$= [R]\left(1 + \frac{[L]}{K_d}\right) \qquad \text{Equation 2-3}$$

Solving for $[R]$ and substituting Equation 2-3 into Equation 2-2 yields:

$$[LR] = \frac{[R_o]\,[L]}{[L] + K_d}, \text{ rearranged to}$$

$$\frac{[LR]}{[R_o]} = \frac{[L]}{[L] + K_d} \qquad \text{Equation 2-4}$$

Note that the left side of this equation, $[LR]/[R_o]$, represents the fraction of all available receptors that are bound to ligand.

Figure 2-1 shows two plots of Equation 2-4 for the binding of two hypothetical drugs to the same receptor. These plots are known as **drug-receptor binding curves.** Figure 2-1A

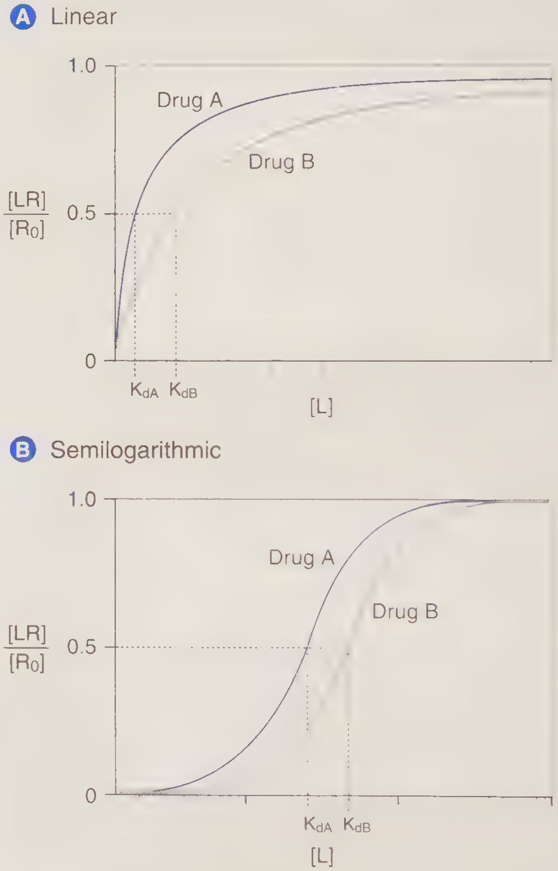


**Figure 2-1. Ligand-Receptor Binding Curves.** **A.** Lineargraphs of drug-receptor binding for two drugs with different values of $K_d$. **B.** Semilogarithmic graphs of the same drug-receptor binding. $K_d$ is the equilibrium dissociation constant for a given drug-receptor interaction—a lower $K_d$ indicates a *tighter* drug-receptor interaction (higher affinity). Because of this relationship, Drug A, which has the lower $K_d$, will bind a greater proportion of total receptors than Drug B at any given drug concentration. Notice that $K_d$ corresponds to the ligand concentration $[L]$ at which 50% of the receptors are bound (occupied) by ligand. $[L]$ is the concentration of free (unbound) ligand (drug), $[LR]$ is the concentration of ligand-receptor complexes, and $R_o$ is the total concentration of occupied and unoccupied receptors. Thus,

$$\frac{[LR]}{[R_o]}$$

is the *fractional occupancy* of receptors, or the fraction of total receptors that are occupied (bound) by ligand.

shows a linear plot, and Figure 2-1B shows the same plot on a semilogarithmic scale. Because drug responses occur over a wide range of doses (concentrations), the semilog plot is often used to display drug-receptor binding data. The two drug-receptor interactions are characterized by different values of $K_d$; in this case, $K_{dA} < K_{dB}$.

Notice from Figure 2-1 that maximum drug-receptor binding occurs when $[LR]$ is equal to $[R_o]$, or $[LR]/[R_o] = 1$. Also notice that, according to Equation 2-4, when $[L] = K_d$, then $[LR]/[R_o] = K_d/2K_d = 1/2$. Thus, *$K_d$ can be defined as the concentration of ligand at which 50% of the available receptors are bound.*

## DOSE-RESPONSE RELATIONSHIPS

The pharmacodynamics of a drug can be quantified by the relationship between the dose (concentration) of the drug and the organism's (patient's) response to that drug. One might intuitively expect the dose–response relationship to be related closely to the drug-receptor binding relationship, and this turns out to be the case for many drug-receptor combinations. Thus, a useful assumption at this stage of discussion is that *the response to a drug is proportional to the concentration of receptors that are bound (occupied) by the drug.* This assumption can be quantified by the following relationship:

$$\frac{\text{response}}{\text{max response}} = \frac{[DR]}{[R_o]} = \frac{[D]}{[D] + K_d} \qquad \text{Equation 2-5}$$

where $[D]$ is the concentration of free drug, $[DR]$ is the concentration of drug-receptor complex, $[R_o]$ is the concentration of total receptors, and $K_d$ is the equilibrium dissociation constant for the drug-receptor interaction. (Note that the right side of Equation 2-5 is equivalent to Equation 2-4, with $[D]$ substituted for $[L]$.) The generalizability of this assumption is examined below.

There are two major types of dose–response relationships—graded and quantal. The difference between the two methods is that graded dose–response relationships describe the effect of various doses of a drug on an individual; whereas, quantal relationships show the effect of various doses of a drug on a population of individuals.

## GRADED DOSE–RESPONSE RELATIONSHIPS

Figure 2-2 shows graded dose–response curves for two hypothetical drugs that elicit the same biological response. The curves are presented on both linear and semilog scales. The curves are similar in shape to those in Figure 2-1, consistent with the assumption that response is proportional to receptor occupancy.

Two important parameters—efficacy and potency—can be deduced from the graded dose–response curve. The **potency ($EC_{50}$)** of a drug *is the concentration at which the drug elicits 50% of its maximal response.* The **efficacy ($E_{max}$)** is *the maximal response produced by the drug.* In accordance with the assumption stated above, efficacy can be thought of as the state at which all receptors are occupied by drug, and, therefore, additional drug will produce no additional response. Some drugs, however, are capable of eliciting a maximal response when fewer than 100% of the drug's receptors are occupied; the remaining receptors can be called "**spare receptors.**" This concept is discussed further in the text that follows. Note again that the graded dose–response curve of Figure 2-2 bears a close resemblance to the drug-receptor binding curve of Figure 2-1, with $EC_{50}$ replacing $K_d$ and $E_{max}$ replacing $R_o$.

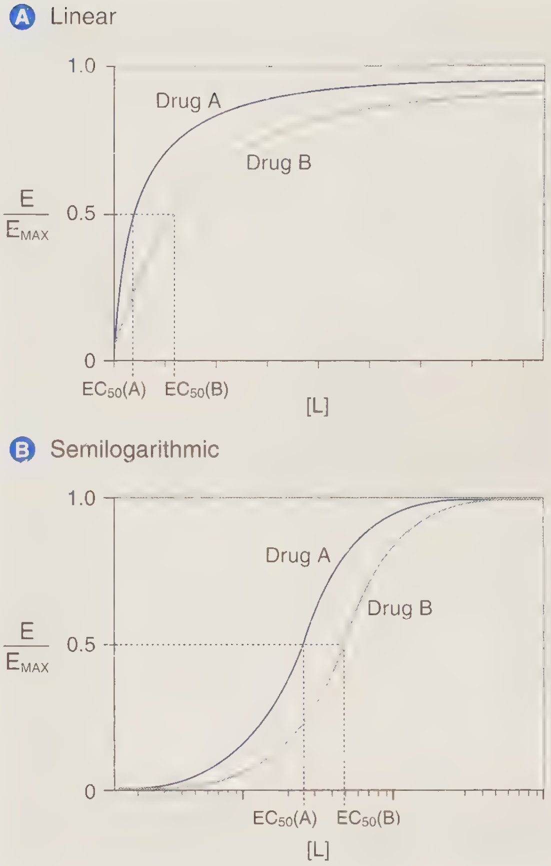


**Figure 2-2. Graded Dose-Response Curves.** Graded dose-response curves demonstrate the effect of a drug as a function of its concentration. **A.** Linear graphs of graded dose-response curves for two drugs. **B.** Semilogarithmic graphs of the same dose-response curves. Note the close resemblance to Figure 2-1: the fraction of occupied receptors $[LR]/[R_o]$ has been replaced by the fractional effect $E/E_{max}$, where $E$ is a quantifiable response to a drug (for example, an increase in blood pressure). $EC_{50}$ is the potency of the drug, or the concentration at which the drug elicits 50% of its maximal effect. In the figure, Drug A has a greater potency than Drug B because it elicits a half-maximal effect at a lower concentration than Drug B. Drugs A and B exhibit the same efficacy (the maximal response to the drug). Note that potency and efficacy are not intrinsically related—a drug can be extremely potent but have little efficacy, and vice versa. $[L]$ is drug concentration, $E$ is effect, $E_{max}$ is efficacy, and $EC_{50}$ is potency.

## QUANTAL DOSE–RESPONSE RELATIONSHIPS

*The quantal dose–response relationship plots the fraction of the population that responds to a given dose of drug against the drug dose.* Quantal dose-response relationships describe the

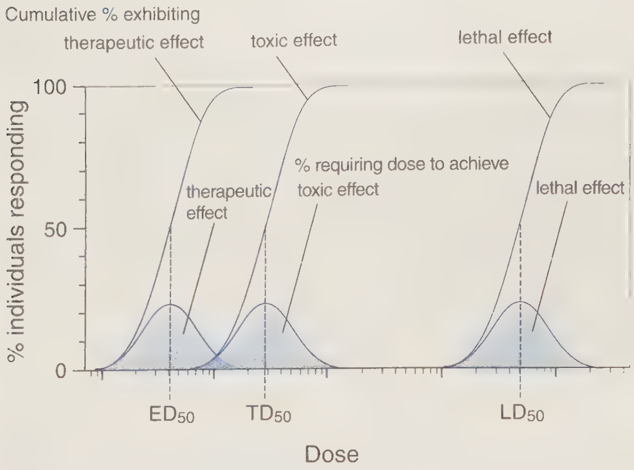


**Figure 2-3. Quantal Dose–Response Curves.** Quantal dose-response curves demonstrate the average effect of a drug, as a function of its concentration, in a population of individuals. Individuals are typically observed for the presence or absence of a response (for example, sleep or no sleep), and this result is then used to plot the percentage of individuals that respond to each dose of drug. Quantal dose–response relationships are useful for predicting the effects of a drug when it is administered to a population of individuals, and for determining population-based toxic doses and lethal doses. These doses are called the $ED_{50}$ (dose at which 50% of subjects exhibit a therapeutic response to a drug), $TD_{50}$ (dose at which 50% of subjects experience a toxic response), and $LD_{50}$ (dose at which 50% of subjects die). Note that $ED_{50}$ is the dose at which 50% of subjects respond to a drug; while $EC_{50}$ (as described in the previous figure) is the dose at which a drug elicits a half-maximal effect in an individual subject.

concentrations of a drug that produce a given effect in a population. Figure 2-3 shows an example of quantal dose-response curves. Because of differences in biological response among a group of individuals, the effects of a drug are seen over a range of doses. The responses are defined as either present or not present (i.e., *quantal,* not *graded*). Examples of this type of response parameter include endpoints such as "sleep/no sleep" or "alive at 12 months/not alive at 12 months"; in contrast, graded dose-response relationships are generated using such scalar responses as change in blood pressure. The goal is to generalize a result to a population, rather than to examine the graded effect of different drug doses on a single individual. Responses that can be examined using the quantal dose–response relationship include effectiveness (therapeutic effect), toxicity (toxic effect), and lethality (lethal effect). The doses that produce these responses in 50% of a population are known as the **median effective dose ($ED_{50}$), median toxic dose ($TD_{50}$),** and **median lethal dose ($LD_{50}$),** respectively.

## DRUG-RECEPTOR INTERACTIONS

Many receptors for drugs can be modeled as having two conformational states that are in reversible equilibrium with one another. These two states are called the active state and the inactive state. Many drugs function as ligands for such receptors and affect the probability that the receptor exists preferentially in one conformation or the other. The pharmacologic properties of drugs are often based on their effects on the state of their cognate receptors. A drug that, upon binding to its receptor, favors the active receptor conformation is called an **agonist;** a drug that favors the inactive conformation is referred to as an **antagonist.** Some drugs do not fit neatly into this simple definition of agonist and antagonist; these include **partial agonists** and **inverse agonists.** The following sections describe these pharmacologic classifications in detail.

## AGONISTS

An agonist is a molecule that binds to a receptor and stabilizes the receptor in its active conformation. When bound by an agonist, a receptor is more likely to be in its active conformation than its inactive conformation. Depending on the receptor, agonists may be drugs or endogenous ligands. A useful model for understanding the relationship between agonist binding and receptor activation is shown in Equation 2-6:

$$\begin{array}{ccc} D + R & \rightleftarrows & D + R^* \\ \downarrow\uparrow & & \downarrow\uparrow \\ DR & \rightleftarrows & DR^* \end{array} \qquad \text{Equation 2-6}$$

where $D$ and $R$ are unbound (free) drug and receptor concentrations, respectively, $DR$ is the concentration of the agonist-receptor complex, and $R^*$ indicates the active conformation of the receptor. For most receptors and agonists, $R^*$ and $DR$ are unstable species that exist only briefly and are quantitatively insignificant compared to $R$ and $DR^*$. Therefore, in most cases, Equation 2-6 simplifies to

$$D + R \rightleftarrows DR^* \qquad \text{Equation 2-7}$$

Note that Equation 2-7 is identical to Equation 2-1, which was used in the analysis of drug-receptor binding. This suggests that, for most receptors, agonist binding is proportional to receptor activation. Some receptors, however, do have limited stability in the $R^*$ and/or $DR$ conformations; in these cases, Equation 2-6 must be revisited (see below).

Equation 2-6 can also be used to illustrate quantitatively the concepts of potency and efficacy. Recall that potency is the agonist concentration required to elicit a half-maximal effect, and efficacy is the maximal effect of the agonist. Assuming that a receptor is not active unless bound to a drug (i.e., that $R^*$ is insignificant compared to $DR^*$), Equation 2-8 provides a quantitative description of potency and efficacy:

$$D + R \;\underset{\text{Potency}}{\underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}}}\; DR \;\underset{\text{Efficacy}}{\underset{k_\beta}{\overset{k_\alpha}{\rightleftarrows}}}\; DR^* \qquad \text{Equation 2-8}$$

Here, $k_\alpha$ is the rate constant for receptor activation and $k_\beta$ is the rate constant for receptor deactivation. This equation demonstrates the relationship between potency ($K_d = k_{off}/k_{on}$) and agonist binding ($D + R \leftrightarrows DR$), as well as the relationship between efficacy ($k_\alpha/k_\beta$) and the conformational change required for activation of the receptor ($DR \leftrightarrows DR^*$). These relationships are intuitive when we consider that more potent drugs are those that have a higher affinity for their receptors (lower $K_d$), and more efficacious drugs are those that cause a higher proportion of receptors to be activated.

## ANTAGONISTS

*An antagonist is a molecule that inhibits the action of an agonist but has no effect in the absence of the agonist.* Figure 2-4 shows one approach to classifying the various types of antagonists. Antagonists can be divided into receptor and nonreceptor antagonists. A **receptor antagonist** binds to either the active site or an allosteric site of a receptor. Binding of antagonist to the active site prevents the binding of agonist to the receptor; whereas, binding of antagonist to an allosteric site prevents the conformational change required for receptor activation. Receptor antagonists can also be divided into **reversible** and **irreversible antagonists;** that is, antagonists that bind to their receptors reversibly and those that bind irreversibly. Figure 2-5 illustrates the general effects of these antagonist types on agonist binding; more detail is provided in the following sections.

A **nonreceptor antagonist** inhibits the ability of an agonist to initiate a response. At the molecular level, this inhibition can occur by binding of the antagonist to a downstream molecule in the activation pathway, by inhibiting the agonist directly (e.g., using antibodies), or by activating a pathway that opposes the action of the agonist. Nonreceptor antagonists can be divided into chemical antagonists and physiological antagonists. **Chemical antagonists** inactivate an agonist before it has the opportunity to act (e.g., by chemical neutralization); **physiologic antagonists** cause a physiologic effect opposite to that induced by the agonist.

This section first discusses competitive receptor antagonists and then noncompetitive receptor antagonists. Finally, nonreceptor antagonists are briefly examined.

### Competitive Receptor Antagonists

*A competitive antagonist binds reversibly to the active site of a receptor.* Unlike an agonist, which also binds to the active site of the receptor, a competitive antagonist does not stabilize the conformational change required for receptor activation. Therefore, the antagonist blocks an agonist from binding to its receptor, while maintaining the receptor in the inactive conformation. Equation 2-9 is a modification of Equation 2-7 that incorporates the effect of a competitive antagonist.

$$AR \rightleftarrows A + D + R \rightleftarrows DR^* \qquad \text{Equation 2-9}$$

In this equation, a fraction of the free receptor molecules ($R$) are unable to form a drug (agonist)-receptor complex ($DR^*$), because receptor binding to antagonist ($A$) results in the formation of an antagonist-receptor complex ($AR$) instead. In effect, the formation of the $AR$ complex sets up a second equilibrium reaction that competes with the equilibrium for agonist-receptor binding. Note that $AR$ is incapable of undergoing a conformational change to the active ($R^*$) state of the receptor.

Quantitative analysis yields the following equation for agonist ($D$) binding to receptor in the presence of a competitive antagonist ($A$):

$$\frac{[DR]}{[R_0]} = \frac{[D]}{[D] + K_d\left(1 + \frac{[A]}{K_A}\right)} \qquad \text{Equation 2-10}$$

Equation 2-10 is similar to Equation 2-4, except that the effective $K_d$ has been increased by a factor of $(1 + [A]/K_A)$, where $K_A$ is the dissociation constant for binding of the antagonist

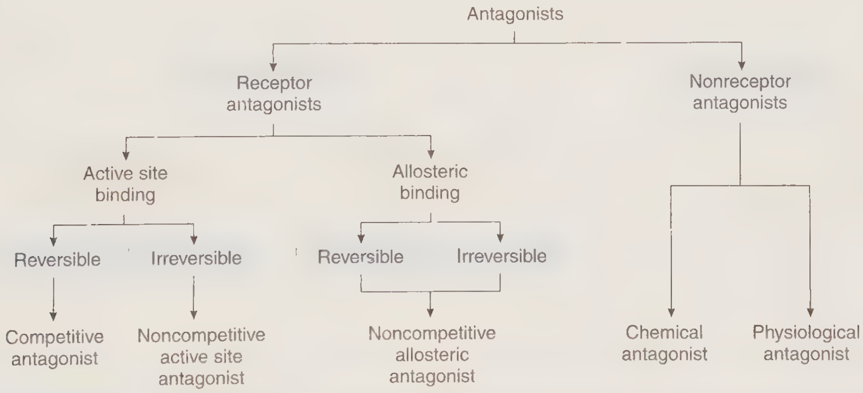
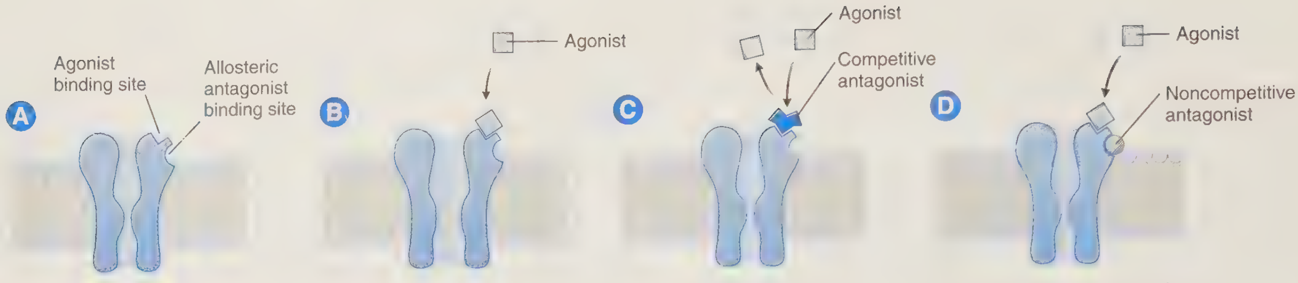


**Figure 2-4. Antagonist Classification.** Antagonists can be categorized based on whether they bind to a site on the receptor (receptor antagonists) or interrupt ligand-receptor signaling by other means (nonreceptor antagonists). Receptor antagonists can bind either to the agonist (active) site or to an allosteric site on the receptor; in either case, they do not affect basal receptor activity (i.e., the activity of the receptor in the absence of agonist). Agonist (active) site receptor antagonists prevent the agonist from binding to the receptor. If the antagonist competes with the ligand for agonist site binding, it is termed a competitive antagonist; high concentrations of agonist are able to overcome competitive antagonism. Noncompetitive agonist site antagonists bind with high affinity or bind covalently to the agonist site, so that even high concentrations of agonist are unable to activate the receptor. Allosteric receptor antagonists bind to the receptor at a site other than the agonist site. They do not compete with agonist for receptor binding, but rather inhibit the receptor from responding to the binding of agonist. In some cases, they may also affect the affinity of the receptor for agonist. High concentrations of agonist are generally unable to reverse the effect of an allosteric antagonist. Nonreceptor antagonists fall into two categories. Chemical antagonists sequester agonist and, thus, prevent the agonist from interacting with the receptor. Physiologic antagonists induce a physiologic response opposite to that of an agonist, but by a molecular mechanism that does not involve the receptor for agonist.



Unbound receptor

Agonist binding

Competitive antagonist binding

Noncompetitive antagonist binding

**Figure 2-5. Types of Receptor Antagonists.** A schematic illustrating the differences between agonist (active) site and allosteric antagonists. **A.** The unbound inactive receptor. **B.** The receptor activated by agonist. Note the conformational change induced in the receptor by agonist binding, for example, the opening of a transmembrane ion channel. **C.** Agonist site antagonists bind to the receptor's agonist site but do not activate the receptor; these agents block agonist binding to the receptor. **D.** Allosteric antagonists bind to an allosteric site (different from the agonist site) and, thereby, prevent receptor activation, even if the agonist is bound to the receptor.

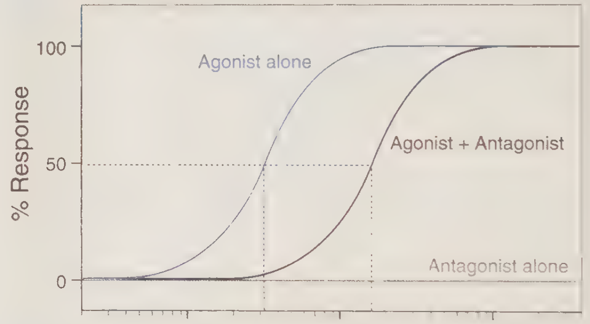
to the receptor (i.e., $K_A = [R][A]/[RA]$). Because an increase in $K_d$ is equivalent to a decrease in potency, *the presence of a competitive antagonist (A) reduces the potency of an agonist (D) by a factor of* $(1 + [A]/K_A)$. Although the potency of an agonist decreases as the concentration of competitive antagonist is increased, the efficacy of the agonist is unaffected. This occurs because the agonist concentration $[D]$ can be increased to counteract ("outcompete") the antagonist, thereby "washing out" or reversing the effect of the antagonist. Figure 2-6A shows the effect of a competitive antagonist on the agonist dose–response relationship. Note that the competitive antagonist has the effect of shifting the agonist dose–response curve to the right, causing a decrease in agonist potency while maintaining agonist efficacy.

Pravastatin, the drug used in the case at the beginning of this chapter to lower the Admiral's cholesterol, is an example of a competitive antagonist. Pravastatin is a member of the HMG CoA reductase inhibitor (statin) class of lipid-lowering drugs. HMG CoA reductase is an enzyme that catalyzes the reduction of HMG CoA, which is the rate-limiting step in cholesterol biosynthesis. The similarity between the chemical structures of statins and HMG CoA allows the statin molecule to bind to the active site of HMG CoA reductase, and thereby to prevent HMG CoA from binding. The decrease in HMG CoA reductase activity decreases endogenous cholesterol synthesis and thereby lowers the patient's cholesterol levels. The inhibition of HMG CoA reductase activity by statins is reversible because no covalent bonds are formed between the statin and the enzyme. For a more detailed discussion of pravastatin and other HMG CoA reductase inhibitors, see Chapter 22 (Pharmacology of Cholesterol and Lipoprotein Metabolism).

## Noncompetitive Receptor Antagonists

Noncompetitive antagonists can bind either to the active site or to an allosteric site of a receptor (Fig. 2-4). A noncompetitive antagonist that binds to the active site of a receptor can bind either covalently or with very high affinity; in either case, the binding is effectively irreversible. Because an irreversibly bound active site antagonist cannot be "outcompeted," even at high agonist concentrations, such an antagonist exhibits noncompetitive antagonism. An allosteric antagonist acts by preventing the receptor from being activated, even when the agonist is bound to the active site. An allosteric antagonist exhibits noncompetitive antagonism, regardless of the reversibility of its binding, because such an antagonist acts not by competing with agonist for binding to the active site, but rather by preventing receptor activation. The reversibility of antagonist binding is, nonetheless, important because the ef-

**A** Competitive antagonist



**B** Noncompetitive antagonist

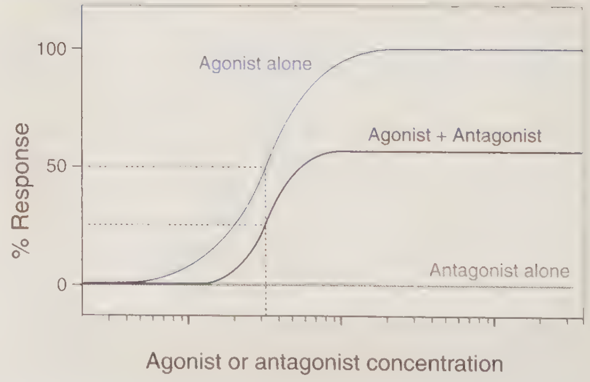


**Figure 2-6. Antagonist Effects on the Agonist Dose-Response Relationship.** Competitive and noncompetitive antagonists have different effects on potency (the concentration of agonist that causes a half-maximal response) and efficacy (the maximal response to an agonist). **A.** A competitive antagonist reduces the potency of an agonist, without affecting agonist efficacy. **B.** A noncompetitive antagonist reduces the efficacy of an agonist. As shown here, most allosteric noncompetitive antagonists do not affect agonist potency.

fect of an irreversible antagonist does not diminish when the free (unbound) drug is eliminated from the body; whereas, the effect of a reversible antagonist can be "washed out" over time as it dissociates from the receptor (see Equation 2-9).

A receptor that is bound by a noncompetitive antagonist can no longer respond to the binding of an agonist. Therefore, the maximal response (efficacy) of the agonist is reduced. A characteristic difference between competitive and noncompetitive antagonists is that *competitive antagonists reduce agonist potency; whereas, noncompetitive antagonists reduce agonist efficacy.* This difference can be explained by considering that a competitive antagonist continuously competes for receptor binding, effectively reducing the receptor's affinity for agonist without limiting the number of available receptors. In contrast, a noncompetitive antagonist removes functional receptors from the system, thereby limiting the number of available receptors. Figures 2-6A and 2-6B compare the effects of a competitive and a noncompetitive antagonist on the agonist dose–response relationship.

Aspirin is one example of a noncompetitive antagonist. This agent irreversibly acetylates cyclooxygenase, the enzyme responsible for generating thromboxane $A_2$ in platelets. In the absence of thromboxane $A_2$ generation, platelet aggregation is inhibited. Because the inhibition is irreversible, and platelets are not capable of synthesizing new cyclooxygenase, the effects of a single dose of aspirin last for 7–10 days (the time required for the bone marrow to generate new platelets), even though the free drug is cleared from the body much more rapidly.

## Nonreceptor Antagonists

Nonreceptor antagonists can be divided into chemical antagonists and physiologic antagonists. A **chemical antagonist** inactivates the agonist of interest by modifying or sequestering it, so that the agonist is no longer capable of binding to and activating the receptor. Protamine is an example of a chemical antagonist; this basic protein binds stoichiometrically to the heparin class of anticoagulants and, thereby, inactivates these agents (see Chapter 21, Pharmacology of Hemostasis and Thrombosis). Because of this chemical antagonism, protamine can be used to terminate the effects of heparin rapidly.

A **physiologic antagonist** most commonly activates or blocks a receptor that mediates a response physiologically opposite to that of the original receptor for agonist. For example, in the treatment of hyperthyroidism, β-adrenergic blocking drugs are used as physiological antagonists to counteract the tachycardic effect of endogenous thyroid hormone. Thyroid hormone does not produce its tachycardic effect via β-adrenergic stimulation, but blocking β-adrenergic stimulation can, nonetheless, relieve the tachycardia caused by hyperthyroidism (see Chapter 8, Adrenergic Pharmacology, and Chapter 25, Pharmacology of the Thyroid Gland).

## PARTIAL AGONISTS

*A partial agonist is a molecule that binds to a receptor at its active site but produces only a partial response, even when all of the receptors are occupied (bound) by the agonist.* Figure 2-7 shows a series of dose–response curves for several full and partial agonists. Each agonist acts by binding to the same site on the muscarinic acetylcholine (ACh) receptor.

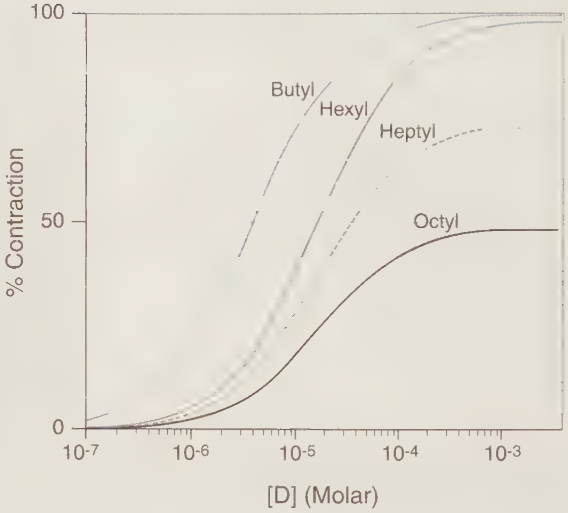


**Figure 2-7. Full and Partial Agonist Dose–Response Curves.** There are many instances in which drugs that all act at the agonist site on the same receptor produce different maximal effects. For example, various alkyl derivatives of trimethylammonium all stimulate muscarinic acetylcholine (ACh) receptors to cause muscle contraction in the gut, but they produce different maximal responses, even when all receptors are occupied. In the figure, the butyl and hexyl trimethylammonium derivatives are full agonists—although they have different potencies, they are capable of eliciting a maximal response. Agonists that produce only a partial response, such as the heptyl and octyl derivatives, are called partial agonists. Note that the dose—response curves of partial agonists plateau at values less than those of full agonists. ACh acts as a full agonist in this system (not shown).

Note that butyl trimethylammonium (TMA) is not only more potent than longer-chain derivatives at stimulating muscle contraction, but also more efficacious than some of the derivatives (e.g., the heptyl and octyl forms) at producing a greater maximum response. For this reason, butyl TMA is a *full agonist* at the muscarinic ACh receptor; whereas, the octyl derivative is a *partial agonist* at this receptor.

One important characteristic of partial agonists is that, in the presence of a full agonist, the partial agonist reduces the maximum response. In this way, the partial agonist acts as a competitive antagonist. For this reason, partial agonists are sometimes called "partial antagonists" or even "mixed agonist-antagonists."

It is interesting to consider how a partial agonist could function if a receptor can exist in only the active or the inactive state. This is an area of current investigation, for which several hypotheses have been proposed. Recall that Equation 2-6 was simplified to Equation 2-7 based on the assumption that *R* and *DR** are much more stable than *R** and *DR*. But what would happen if a drug (call it a partial agonist) could stabilize *DR* as well as *DR**? In that case, addition of the partial agonist would result in stabilization of some receptors in the *DR* form and some receptors in the *DR** form. At full receptor occupancy, some receptors would be in the active state and some in the inactive state, and the efficacy of the drug would be reduced compared to that of a full agonist (which stabilizes only *DR**). In this formulation, a pure antagonist binds preferentially to the inactive state of the receptor, a full agonist binds preferentially to the active state of the receptor, and a partial agonist binds with similar affinity to both the active and inactive states of the receptor.

A second hypothesis for the action of partial agonists is that a receptor may have multiple *DR** conformations. Each conformation may possess a different activity, so that only a subset of the maximum possible pharmacologic effect is observed when a partial agonist binds to the receptor. This may be the case with the so-called selective estrogen receptor modulators (SERMs) such as raloxifene and tamoxifen (see Chapter 27, Pharmacology of Reproduction). Raloxifene acts as a partial agonist at estrogen receptors in bone. The crystal structure of raloxifene bound to estrogen receptor, when compared to that of estrogen bound to estrogen receptor, reveals that the side chain of raloxifene inhibits an α helix of the estrogen receptor from aligning in the active site. This may result in inhibition of some effects of the estrogen receptor, while maintaining other effects. At a physiologic level, this would be observed as partial agonism.

Another example of a partial agonist is pindolol, a drug usually classified as a β-adrenergic antagonist (see Chapter 8). Pindolol demonstrates partial agonist properties, and this drug may be of clinical value because of the intermediate response that it produces. Although resting heart rate and blood pressure are not reduced as much by pindolol as by other pure β-adrenergic antagonists, pindolol does inhibit the potentially dangerous heart rate and blood pressure increases that would otherwise occur with sympathetic stimulation (e.g., with exercise) in patients with cardiovascular disease.

## INVERSE AGONISTS

The action of inverse agonists can be understood by again considering Equation 2-6. As noted above, in some cases receptors can have inherent stability in the *R** as well as the *R* state. In these cases, there is intrinsic activity ("tone") of the receptor system, even in the absence of an endogenous ligand or an exogenously administered agonist. *An inverse agonist acts by abrogating this intrinsic activity of the free (unoccupied) receptor.* One way inverse agonists could function is by binding to and stabilizing the receptor in the *DR* (inactive) form. This would have the effect of deactivating receptors that had existed in the *R** form in the absence of drug. The relative importance in human physiology of receptors that exist in the *R** state for a significant period of time is currently under investigation.

It is important to consider the similarities and differences between the actions of inverse agonists and competitive antagonists. Both types of drug act to reduce the activity of a receptor. In the presence of a full agonist, both competitive antagonists and inverse agonists act to reduce agonist potency. Recall, however, that a competitive antagonist has no effect in the absence of agonist; whereas, an inverse agonist deactivates receptors that are constitutively active in the absence of agonist. Using Equations 2-6 through 2-9 as models, these concepts can be summarized by stating that *full agonists stabilize DR*, partial agonists stabilize both DR and DR* (or alternate forms of DR*), inverse agonists stabilize DR, and competitive antagonists "stabilize" R (or AR) by preventing full, partial, and inverse agonists from binding to the receptor.*

## SPARE RECEPTORS

Recall that the initial discussion of drug-receptor binding curves assumed that 100% receptor occupancy is required for an agonist to exert its maximal effect. Now, consider the possibility that a maximal response could be achieved with less than 100% receptor occupancy. Figure 2-8 shows an example of a drug-receptor binding curve and a dose–response curve that illustrate this situation. In this example, a maximal effect is achieved at a lower dose of agonist than that required

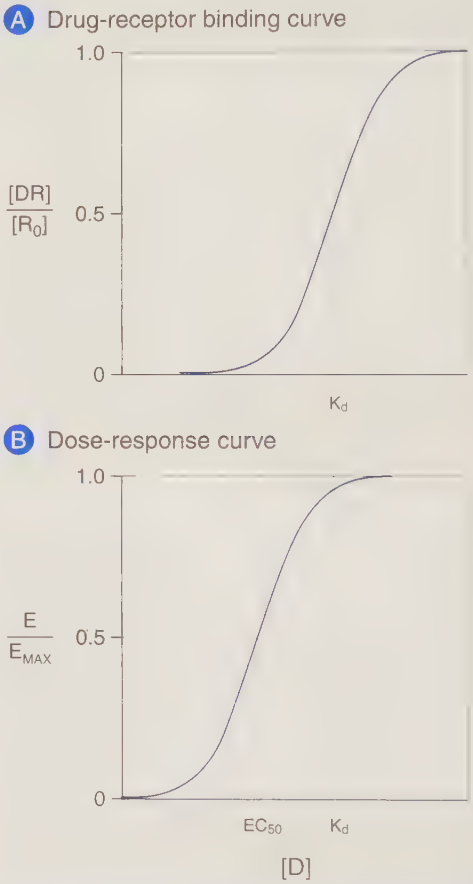


**Figure 2-8. Comparison Between a Drug-Receptor Binding Curve and a Dose-Response Curve in the Presence of Spare Receptors.** In the absence of spare receptors, there often exists a close correlation between a drug-receptor binding curve and a dose-response curve—the binding of additional drug to the receptor causes an incremental increase in response, and $EC_{50}$ is approximately equal to $K_d$. In situations with spare receptors, however, fewer than half the total receptors must be occupied to elicit a half-maximal response (the term "spare" implies that it is not necessary to occupy every receptor with drug to elicit a full response). **A.** Drug-receptor binding curve. **B.** Dose-response curve for the same drug, in the presence of spare receptors. Note that the maximal response occurs at a lower agonist concentration than does maximal binding, and that $EC_{50} < K_d$. These two relationships confirm the presence of spare receptors. *D* is drug, *R* is receptor, and $[DR]/[R_o]$ is fractional receptor occupancy. *E* is response (effect), $E_{max}$ is maximal response (efficacy), and $E/E_{max}$ is fractional response. $EC_{50}$ is potency, and $K_d$ is the equilibrium dissociation constant for drug-receptor binding.

for receptor saturation, that is, the $EC_{50}$ is less than the $K_d$ for this system. This type of discrepancy between the drug-receptor binding curve and the dose–response curve signifies the presence of spare receptors. At least two possible molecular mechanisms are thought to be responsible for the spare receptor phenomenon. First, the receptor could remain activated after the agonist departs, allowing one agonist molecule to activate several receptors simultaneously. Second, the cell signaling pathways described in Chapter 1 could allow for significant amplification of a relatively small signal, and activation of only a few receptors could be sufficient to produce a maximal response. This is true, for example, with many G protein-coupled receptors; activation of a single $G\alpha_s$ molecule can stimulate adenylyl cyclase to catalyze the formation of dozens of molecules of cAMP.

The presence of spare receptors alters the effect of a noncompetitive antagonist on the system. At low antagonist concentrations, the noncompetitive antagonist binds receptors that are not required to produce a maximal response; therefore, the efficacy of the agonist is not decreased. The potency of the agonist is affected, however, because potency is proportional to the fraction of available receptors that must be occupied to produce a 50% response. A noncompetitive antagonist reduces the number of available receptors, thereby increasing the fraction of receptors that must be bound at any agonist dose to produce the same response. At high antagonist concentrations, the noncompetitive antagonist binds not only the "spare" receptors but also receptors that are required to produce a maximal response, and the efficacy as well as the potency of the agonist is decreased. Figure 2-9 illustrates this concept.

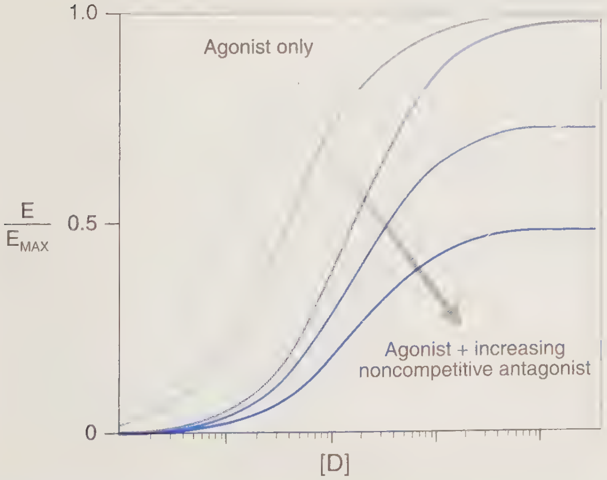


**Figure 2-9. Effect of a Noncompetitive Antagonist on the Agonist Dose–Response Curve in the Presence of Spare Receptors.** In a system without spare receptors, a noncompetitive antagonist causes efficacy to decrease (see Figure 2-6B). In a system with spare receptors, however, potency is decreased, but efficacy is unaffected at low concentrations of the antagonist because a sufficient number of unoccupied receptors is available to generate a maximal response. As increasing concentrations of antagonist bind noncompetitively to more and more receptors, the antagonist eventually occupies all of the "spare" receptors, and efficacy is also reduced.

## CONCEPTS IN THERAPEUTICS

### THERAPEUTIC INDEX AND THERAPEUTIC WINDOW

The **therapeutic window** is the range of doses (concentrations) of a drug that elicits a therapeutic response, without unacceptable side effects (toxicity), in a population of patients. Plasma levels of a drug that has a small therapeutic window must be monitored closely to maintain effective dosing without exceeding the level that could produce toxicity. The next chapter discusses some of the techniques used in clinical therapeutics to maintain plasma concentrations of drugs within the therapeutic window.

The therapeutic window can be quantified by the **therapeutic index (TI)** (sometimes called the therapeutic ratio), commonly defined as:

$$\text{Therapeutic Index (TI)} = \frac{TD_{50}}{ED_{50}} \qquad \text{Equation 2-11}$$

where $TD_{50}$ is the dose of the drug that causes a toxic response in 50% of the population, and $ED_{50}$ is the dose of the drug that is therapeutically effective in 50% of the population. The TI provides a single number that quantifies the relative margin of safety of a drug in a population of people. A large TI represents a large (or "wide") therapeutic window (for example, a thousand-fold difference between the therapeutic and toxic doses), and a small TI represents a small (or "narrow") therapeutic window (for example, a twofold difference between the therapeutic and toxic doses). The potential toxicities associated with the use of heparin and tPA in the case at the beginning of this chapter are indicated by the low TIs of these drugs. Aspirin's high TI is indicative of its relative safety. Note that the pharmacologic effect of heparin was monitored periodically in the case; whereas, aspirin could be administered without the need to monitor its plasma drug level.

## ■ *Conclusion and Future Directions*

Pharmacodynamics is the quantitative study of the effects of drugs on the body. Several tools have been developed to compare the efficacy and potency of drugs, including the graded and quantal dose-response relationships. The former is used to examine the effects of varying drug doses on an individual; whereas, the latter is used to examine the effects of varying drug doses on a population. The therapeutic window and therapeutic index are used to compare the concentrations of drugs that produce therapeutic effects and toxic (side) effects.

In the study of pharmacodynamics, drugs can be divided into two broad classes—agonists and antagonists. Most agonists cause a receptor to maintain a conformation in the active state; whereas, antagonists stabilize the receptor in the inactive state. Antagonists are further divided according to the molecular location of their effect (i.e., receptor or non-

**TABLE 2-1** Summary of Agonist and Antagonist Action

CLASSES OF AGONISTS

| AGONIST CLASS | ACTION |
|---|---|
| Full agonist | Activates receptor with maximal efficacy |
| Partial agonist | Activates receptor, but not with maximal efficacy |
| Inverse agonist | Inactivates constitutively active receptor |

CLASSES OF ANTAGONISTS

| ANTAGONIST CLASS | EFFECTS ON AGONIST POTENCY | EFFECTS ON AGONIST EFFICACY | ACTION |
|---|---|---|---|
| Competitive antagonist | Yes | No | Binds reversibly to active site of receptor |
| Noncompetitive active site antagonist | No | Yes | Binds irreversibly to active site of receptor |
| Noncompetitive allosteric antagonist | No | Yes | Binds reversibly or irreversibly to site other than active site of receptor |

receptor), the site at which they bind to the receptor (i.e., active site or allosteric site), and the mode of their binding to the receptor (i.e., reversible or irreversible). Table 2-1 provides a summary of the various types of agonists and antagonists presented in this chapter.

The information presented in this chapter is used repeatedly throughout this book and throughout one's career as a health professional. Whenever drugs are compared according to potency or efficacy, or the appropriate dose of a drug for a specific patient must be determined, a working knowledge of pharmacodynamics is essential.

## *Suggested Readings*

Pratt WB, Taylor P, eds. Principles of Drug Action: The Basis of Pharmacology, 3rd Ed. New York: Churchill Livingstone, 1990. (*Contains an in-depth discussion of pharmacodynamics.*)

Berg JM, Tymoczko JL, Stryer L. Biochemistry, 5th Ed. New York: WH Freeman and Company, 2002. (*Discusses the structural basis for protein–protein interactions.*)

Leff P. The two-state model of receptor activation. Trends Pharmacol Sci 1995;16:89–97. (*Provides the theoretical grounding for Equation 2-6; discusses quantitative treatment of drug-receptor interactions.*)

# 3

# Pharmacokinetics

John C. LaMattina and David E. Golan

**Introduction**
**Case**
**Physiologic Barriers**
  Cell Membrane
    *Traversing the Membrane*
    *Membrane Diffusion*
  Central Nervous System
**Absorption**
  Administration Routes and Rationale
    *Enteral*
    *Parenteral*
    *Mucous Membrane*
    *Transdermal*
  Local Factors Affecting Absorption
**Distribution**
  Volume of Distribution
    *Plasma Protein Binding*
  Modeling the Kinetics and Thermodynamics of Drug Distribution
**Metabolism**
  Phase I
  Phase II
**Excretion**
  Renal Excretion
  Biliary Excretion
**Clinical Applications of Pharmacokinetics**
  Clearance
    *Metabolism and Excretion Kinetics*
  Half-Life
    *Factors Altering Half-Life*
  Therapeutic Dosing and Frequency
    *Loading Dose*
    *Maintenance Dose*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Even the most promising of pharmacologic therapies will fail in clinical trials if the drug is unable to reach its target organ at a concentration sufficient to have a beneficial effect. Many of the characteristics that render the human body resistant to harm by foreign invaders and toxic substances also limit the ability of modern drugs to combat pathologic processes within the patient. An appreciation of the many factors that affect a drug's ability to act within a patient, and the dynamic nature of these factors over time, are vitally important to the physician in the clinic.

All drugs must meet certain minimal requirements to achieve clinical effectiveness. A successful drug must be able to cross the physiologic barriers that exist to limit the access of foreign substances into the body. Drug **absorption** may occur by a number of mechanisms that are designed either to exploit or to breach these barriers. Following absorption, the drug uses **distribution** systems within the body, such as the blood and lymphatic vessels, to reach its target organ in an appropriate concentration. The drug's ability to access its target is also limited by several processes within the patient. These processes fall broadly into two categories: **metabolism,** in which the body inactivates the drug through enzymatic degradation (primarily in the liver), and **excretion,** in which the drug is excreted from the body (primarily in the kidneys, liver, and feces). This chapter presents a broad overview of the pharmacokinetic processes of absorption, distribution, metabolism, and excretion (Fig. 3-1), with a conceptual emphasis on basic principles that, when applied to an unfamiliar scenario, should enable the student or physician to understand the pharmacokinetic basis for drug therapy.

## ■ Case

Michael Walsh is a 58-year-old technology consultant who makes frequent trips abroad as part of his job in the telecommunications industry. On a recent trip, he flew to Turkey for a week. On the last night of his excursion, Mr. Walsh went to a large dinner featuring a number of shish kebabs and other foods he did not often eat. The next day, he developed profuse, watery, and foul-smelling diarrhea. Before this episode, Mr. Walsh had been relatively

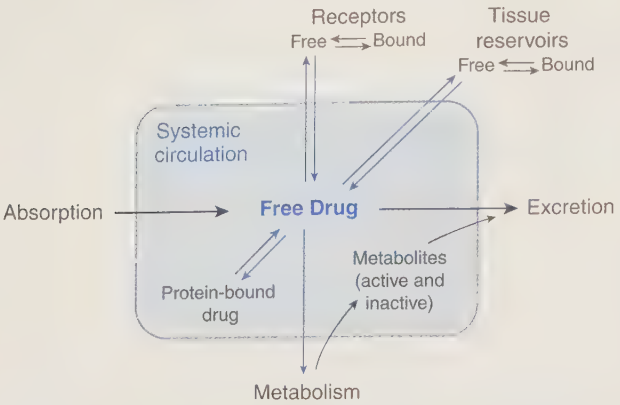


**Figure 3-1. Drug Absorption, Distribution, Metabolism, and Excretion (ADME).** The basic principles of pharmacokinetics affect the amount of free drug that ultimately reaches the target site. To elicit an effect on its target, a drug must be absorbed and then distributed to its target before being metabolized and excreted. At all times, free drug in the systemic circulation is in equilibrium with tissue reservoirs, plasma proteins, and the target site (which usually consists of receptors); only the fraction of drug that successfully binds to specific receptors will have a pharmacologic effect. Note that metabolism of drug can result in both inactive and active metabolites; active metabolites may also be able to exert a pharmacologic effect, either on the target receptors or sometimes on other receptors.

healthy except for chronic atrial fibrillation, for which he takes warfarin. The physician made a diagnosis of traveler's diarrhea, and prescribed a 7-day course of trimethoprim-sulfamethoxazole. Four days later, Mr. Walsh noted multiple hematomas across his trunk and extremities. Laboratory studies showed a markedly prolonged prothrombin time that was indicative of supratherapeutic (toxic) levels of warfarin in Mr. Walsh's blood.

## QUESTIONS

- 1. How does a patient with well-established therapeutic blood levels of a chronic medication suddenly develop clinical manifestations of drug toxicity?
- 2. Could this situation have been avoided? If so, how?

## PHYSIOLOGIC BARRIERS

A drug must overcome physical, chemical, and biological barriers to reach its molecular and cellular sites of action. The epithelial cell lining of the gastrointestinal tract and other mucous membranes are one sort of barrier; additional barriers are encountered after the drug enters the bloodstream and lymphatics. Most drugs must distribute from the bloodstream into local tissues, a process that may be impeded by such structures as the blood–brain barrier. Typically, drugs leave the intravascular compartment at the level of the postcapillary venules, where there are larger gaps between the endothelial cells through which the drug can pass. Drug distribution occurs mainly through passive diffusion, the rate of which is affected by local ionic and cellular conditions. This section describes the major physical, chemical, and biological barriers presented by the body and the properties of drugs that favor or disfavor the drugs' ability to overcome these barriers.

## CELL MEMBRANE

All human cells are limited by a lipid bilayer membrane. The membrane contains a hydrophobic lipid core and presents a hydrophilic surface to the aqueous extracellular and intracellular environments. The major lipid components of biological membranes are amphiphilic molecules including phospholipids, cholesterol, and other minor species. The hydrophilic phosphate-containing head groups of phospholipids, and the polar hydroxyl groups of cholesterol are exposed at the outer and inner membrane surfaces, while the hydrophobic tails of the lipids face the interior of the membrane. In addition to lipid components, biologic membranes contain many different proteins. Some of these proteins are exposed only at the extracellular or intracellular membrane surface; some (called transmembrane proteins) penetrate through the lipid bilayer and are exposed at both membrane surfaces. This design has important implications for drug therapy. For a drug either to affect intracellular targets or to pass through a cell, it must be able to cross at least one, and usually several, biological membranes.

### Traversing the Membrane

The hydrophobic core of a biologic membrane presents the major barrier to drug transport. Nonpolar molecules, such as steroids, are often able to diffuse easily through the membrane. Many therapeutic drugs are sufficiently large and polar, however, as to render this an ineffective method of transport across the membrane. Some transmembrane proteins provide channels or pores through which polar drugs and molecules can pass. Other drugs are designed either to act directly on the cell surface (e.g., by binding to a specific cell surface receptor), or to enter the cell through more specialized transmembrane carrier proteins that facilitate the passage of larger molecules. These carrier proteins are often specific for the drug and related endogenous molecules; upon binding of the drug to the extracellular surface of the protein, the protein undergoes a conformational change that may be energy independent (called **facilitated diffusion**) or require the input of energy (called **active transport**). This conformational change allows the bound drug access to the interior of the cell, where the drug molecule is released from the transmembrane protein. Some drugs bind to specific cell surface receptors and trigger a process called endocytosis, in which the cell membrane involutes around the molecule to form a closed cavity or vesicle from which the drug is subsequently released into the interior of the cell.

### Membrane Diffusion

In the absence of any other factors, a drug will enter a cell until the intracellular and extracellular concentrations of the drug are equivalent. The rate of diffusion depends on the concentration gradient of the drug across the membrane and on the thickness, area, and permeability of the membrane. Fick's law of diffusion states that the net drug flux across the membrane is:

$$\text{Flux} = \frac{(C2 - C1) \times (\text{Area} \times \text{Permeability})}{\text{Thickness}_{\text{membrane}}} \qquad \text{Equation 3-1}$$

where $C1$ and $C2$ are the intracellular and extracellular concentrations of the drug, respectively. This definition applies to an ideal situation where there is an absence of complicating factors such as ionic, pH, and charge gradients across the membrane. In vivo, however, these additional factors affect the tendency of a drug to enter cells. For example, a higher concentration of drug outside the cell would ordinarily tend to favor net drug entry into the cell, but if both the cell interior and the drug are negatively charged, then net drug entry into the cell may be impeded. A positively charged or basic drug would show an opposite electrical tendency, and entry into the cell would be favored.

Net diffusion of acidic and basic drugs across the lipid bilayer membrane is also affected by a charge-based phenomenon known as **pH trapping**. The extent of drug trapping on one side of the membrane is determined by the drug's acid dissociation constant ($pK_a$) and by the pH gradient across the membrane. For example, such weakly acidic drugs as phenobarbital and aspirin are protonated (and, therefore, electrically neutral) in the highly acidic environment of the stomach; these nonionic forms of the drugs are more likely to pass through the lipid bilayers of the gastric mucosa, speeding the drugs' absorption (Fig. 3-2). In the more basic environment of the plasma, however, weakly acidic drugs are deprotonated and are, therefore, less likely to diffuse back across the gastric mucosa. This effectively traps the drug within the plasma.

The $pK_a$ of a drug represents the pH value at which one-half of the drug is present in its ionic form, and indicates the pH above and below which pH trapping can affect the ability of the drug to traverse biological membranes. The Henderson–Hasselbach Equation describes the relationship between the $pK_a$ of an acidic or basic drug $A$ and the pH of the biological medium containing the drug:

$$pK_a = \text{pH} + \log \frac{[HA]}{[A^-]} \qquad \text{Equation 3-2}$$

where $HA$ is the protonated form of drug $A$. For example, consider the hypothetical case of a weakly acidic drug with a $pK_a$ of 4. In the stomach, which has a pH of approximately 1, Equation 3-2 becomes:

$$pK_{a_{\text{drug}}} = \text{pH}_{\text{stomach}} + \log \frac{[HA]}{[A^-]},$$

which simplifies to:

$$3 = \log \frac{[HA]}{[A^-]},$$

and finally:

$$1{,}000 = \frac{[HA]}{[A^-]}.$$

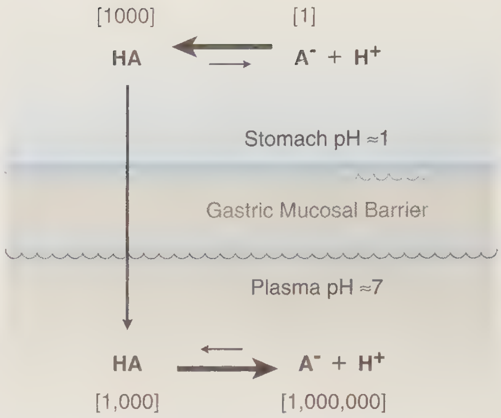


**Figure 3-2. pH Trapping Across Lipid Bilayers.** In the example shown, consider a hypothetical drug with pKa = 4. Although this drug is a weak acid, in the highly acidic environment of the stomach, it is largely protonated. If the stomach pH is approximately 1, then for every 1,001 molecules of drug, 1,000 molecules are protonated (and neutral) and only 1 is deprotonated (and negatively charged). The protonated, neutral form of the drug is able to diffuse across the gastric mucosal barrier into the blood. Assume, however, that the blood plasma has a pH of approximately 7 (it is actually 7.4). Because the drug has a pKa of 4, the vast majority of drug now exists in the deprotonated (negatively charged) form; for every 1,001 molecules of drug, only 1 molecule is protonated (and neutral), while 1,000 molecules are deprotonated (and negatively charged). The negatively charged form of the drug is no longer able to diffuse across the lipid bilayers of the gastric mucosa, and the drug is effectively trapped in the plasma.

Therefore, in the stomach, the protonated (neutral) form of the drug exists in 1,000 times the concentration of the deprotonated (charged) form; 99.9% of the drug is in the neutral form. A similar calculation for the plasma, in which the pH is approximately 7 (plasma pH is actually 7.4), would demonstrate that the situation is reversed, with 99.9% of the drug in the deprotonated (charged) form (see Fig. 3-2).

## CENTRAL NERVOUS SYSTEM

The central nervous system (CNS) presents special challenges to pharmacologic therapy. Unlike other anatomical regions, the CNS (and, interestingly, the testis) is particularly well insulated from foreign substances. The **blood–brain barrier** uses specialized tight junctions to prevent the passive diffusion of most drugs from the systemic to the cerebral circulation. Therefore, drugs designed to act in the CNS must either be sufficiently hydrophobic to traverse biological membranes easily or use existing transport proteins in the blood–brain barrier to penetrate central structures. Hydrophilic drugs that fail to target facilitated or active transport proteins in the blood–brain barrier cannot penetrate the CNS. The blood–brain barrier can be bypassed using intrathecal drug infusion, in which drugs are delivered directly into the cerebrospinal fluid (CSF). Although this approach can be used to treat infectious or carcinomatous meningitis, the intrathecal route is impractical for drugs that must be taken regularly by the patient.

## ABSORPTION

The human body presents formidable obstacles to microorganisms that would seek to invade it. The integument, with its keratinized outer layer and the defensins found within its epithelium, presents an inhospitable surface for invasion. The mucous membranes, while presenting a more feasible penetration point, are also replete with nonspecific defense mechanisms, including mucociliary clearance in the trachea, lysozyme secretion from lacrimal ducts, acid in the stomach, and base in the duodenum. These nonspecific mechanisms must also be overcome by any drug, or the amount of the drug available to the target organ, referred to as the drug's **bioavailability** (also known as the fraction absorbed), will never be sufficiently high for the drug to be efficacious. This concept is often quantified numerically as:

$$\text{Bioavailability} = \frac{\text{Quantity of drug reaching systemic circulation}}{\text{Quantity of drug administered}} \qquad \text{Equation 3-3}$$

This definition of bioavailability is based on the fact that most drugs reach their molecular and cellular sites of action directly from the systemic circulation. Intravenously administered drugs are injected directly into the systemic circulation; for these drugs, the quantity administered is equivalent to the amount reaching the systemic circulation and the bioavailability is, by definition, 1.0. In contrast, incomplete absorption and hepatic metabolism usually cause the bioavailability of an orally administered drug to be less than 1.0 (Fig. 3-3).

A drug may show exceptional in vitro efficacy, but this efficacy may not be comparable to the drug's in vivo effectiveness because of decreased bioavailability. The method of introducing the drug into the body, the chemical form of the drug, and a number of patient-specific factors such as gastrointestinal enzymes and hepatic metabolism will determine the drug's bioavailability. In most cases, a drug may reach its target organ only in a form that is soluble in aqueous solution. Although medicinal chemists can dissolve a drug in ethanol or some other solvent in order to perform preclinical testing of the drug, this mode of administration is ordinarily not feasible in humans. Drugs that are soluble in aqueous solution at physiological pH can often be administered by the oral route; whereas, those that are soluble only in oil must typically rely on other routes. Both the physical state of a drug and other factors outlined below affect the route(s) by which the drug can be administered.

## ADMINISTRATION ROUTES AND RATIONALE

A drug can be absorbed only if the barriers presented by the body can be circumvented. Each new drug is designed and tested in a dosage form that is administered by a specific route. This route of administration enables the drug to penetrate barriers, often taking advantage of transport molecules and other mechanisms that permit the drug to enter from an exterior compartment into body tissues. This section discusses the advantages and disadvantages of drug administration by enteral (oral), parenteral, mucous membrane, and transdermal routes (Table 3-1).

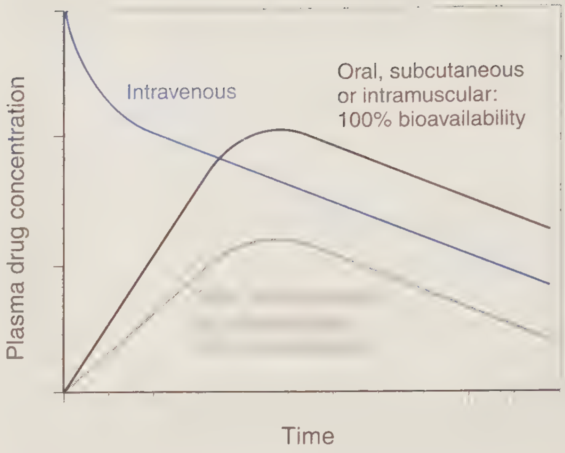


**Figure 3-3. Bioavailability Following Administration of a Single Dose of Drug.** An intravenously administered drug is immediately available in the circulation. The drug is then distributed to other body compartments (see Fig. 3-7) and eliminated by first-order kinetics (see Fig. 3-6). In contrast, other routes of administration (e.g., oral, subcutaneous, and intramuscular) demonstrate slower entry into the bloodstream. In addition, other routes of administration must take into account bioavailability—for example, many orally administered drugs are incompletely absorbed or undergo first-pass metabolism in the liver. If a drug has 100% bioavailability, the total amount of drug reaching the systemic circulation will be the same for all routes of drug administration, but nonintravenous routes will require a longer period of time to reach a maximal concentration of drug in the plasma. If the bioavailability of an oral, subcutaneous, or intramuscular dosage form is less than 100%, then the dose of the drug would have to be increased in order for the total amount of drug reaching the systemic circulation to be the same as that of an intravenous dose.

### Enteral

Enteral drug administration, or the administration of a drug by mouth, is the simplest of drug routes. By administering the drug orally, the enteral route exploits existing weaknesses in human barrier defenses. This route provides many advantages for the patient, but exposes the drug to harsh environments that may limit its absorption. A patient may easily self-administer an enteral drug, requiring no skilled medical care. Enteral drug administration is less likely than other methods to introduce systemic infection as a complication of treatment. Drugs administered by this route are exposed to harsh acidic (stomach) and basic (duodenum) environments, however, so adequate absorption often requires that the drug is stable in these environments.

An orally administered drug must also be stable during its absorption across the gastrointestinal tract epithelium. Gastrointestinal epithelial cell junctions make paracellular transport across an intact epithelium difficult. Instead, ingested

**TABLE 3-1** Routes of Drug Administration

| ROUTE | ADVANTAGES | DISADVANTAGES |
|---|---|---|
| Enteral (e.g., aspirin) | Simple, inexpensive, convenient, painless, no infection | Drug exposed to harsh GI environments and first-pass metabolism, requires GI absorption, slow delivery to site of pharmacologic action |
| Parenteral (e.g., morphine) | Rapid delivery to site of pharmacologic action, highest bioavailability, not subject to first-pass metabolism or harsh GI environments | Irreversible, infection, pain, fear, skilled personnel required |
| Mucous membrane (e.g., beclomethasone) | Rapid delivery to site of pharmacologic action, not subject to first-pass metabolism or harsh GI environments, often painless, simple, convenient, low infection, direct delivery to affected tissues (e.g., lung) possible | Few drugs available to administer via this route |
| Transdermal (e.g., nicotine) | Simple, convenient, painless, excellent for continuous or prolonged administration, not subject to first-pass metabolism or harsh GI environments | Requires highly lipophilic drug, slow delivery to site of pharmacologic action, may be irritating |

substances (such as drugs) must often traverse the cell membrane at the apical surface, and again at the basal surface, before entering the bloodstream. The efficiency of this process is determined by drug size and hydrophobicity, and sometimes by the presence of carriers through which the drug may enter and/or exit the cell. *In general, hydrophobic and neutral drugs cross cell membranes more efficiently than do hydrophilic or charged drugs, unless the membrane contains a carrier molecule that permits the passage of hydrophilic substances.*

Upon traversing the gastrointestinal epithelium, a drug is carried by the portal system to the liver before entering the systemic circulation. While the portal circulation serves to protect individuals from the systemic effects of ingested toxins by delivering these substances to the liver for detoxification, this system may complicate drug delivery. All orally administered drugs are subjected to "**first-pass metabolism**" in the liver. In this process, liver enzymes may inactivate a fraction of the ingested drug. Any drug that exhibits significant first-pass metabolism must be administered in a quantity sufficient to ensure that an effective concentration of active drug exits the liver into the systemic circulation, from which it can reach the target organ. Nonenteral routes of drug administration are not subjected to first-pass liver metabolism. Finally, a fraction of an orally administered drug may pass through the digestive tract without being absorbed; this also must be accounted for in considering oral dosing regimens.

## Parenteral

Parenteral drug administration, in which a drug is introduced directly across the body's barrier defenses into the systemic circulation or some other tissue space, immediately overcomes barriers that can limit the effectiveness of orally administered drugs. Drugs can be administered parenterally into vascularized tissue or injected directly into the bloodstream or cerebrospinal fluid (Table 3-2). Tissue administration results in a rate of onset of drug action that differs among the various body tissues, depending on the rate of blood flow to the tissue. Subcutaneous (SC) administration of drug into poorly vascularized adipose tissue results in a slower onset of action than does injection into well-vascularized intramuscular (IM) spaces. Drugs that are soluble only in oil-based solution are often administered intramuscularly. Direct introduction of drug into the venous [intravenous (IV)] or arterial [intra-arterial (IA)] circulation, or into the cerebrospinal fluid [intrathecal (IT)], results in the drug reaching its target organ the fastest. Intravenous injection is not typically limited in the amount of drug that can be delivered, as subcutaneous and intramuscular injections may be. Continuous intravenous

**TABLE 3-2** Routes of Parenteral Drug Administration

| PARENTERAL ROUTE | ADVANTAGES | DISADVANTAGES |
|---|---|---|
| Subcutaneous (e.g., xylocaine) | Slow onset, may be used to administer oil-based drugs | Slow onset, small volumes |
| Intramuscular (e.g., haloperidol) | Intermediate onset, may be used to administer oil-based drugs | Can affect lab tests (creatine kinase), intramuscular hemorrhage, painful |
| Intravenous (e.g., morphine) | Rapid onset, controlled drug delivery | Peak-related drug toxicity |
| Intrathecal (e.g., methotrexate) | Bypasses blood–brain barrier | Infection, highly skilled personnel required |

infusions also have the advantage of controlled drug delivery, so that the dose of drug delivered can be altered at any time.

Although the physical properties of certain drugs (e.g., size and hydrophobicity) may necessitate a particular route of drug administration, the clinician will often have a choice among routes. Parenteral administration may be associated with several potential disadvantages that must be considered before deciding to use this route. Parenteral administration introduces an increased risk of infection and typically requires administration by a health care professional. The rate of onset of parenterally administered drugs is often rapid, potentially resulting in increased morbidity when such drugs are administered too rapidly or in incorrect doses. These disadvantages must be weighed against the advantages of parenteral administration and the strength of the indication for pharmacologic therapy.

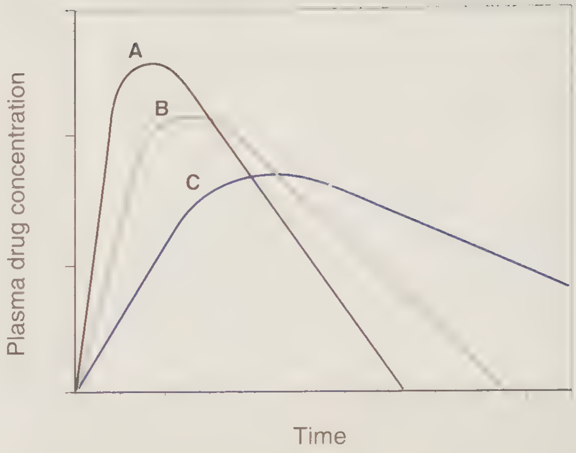


**Figure 3-4. Effect of Rate of Absorption on Peak Concentration of Drug in Plasma and Duration of Drug Action.** The duration of action and peak plasma concentration of a drug can be affected markedly by the drug's absorption rate. In this example, three drugs with identical bioavailability, volume of distribution, and clearance are administered in identical doses. The drugs demonstrate different rates of absorption—drug A is absorbed quickly, drug C is absorbed slowly, and drug B's absorption rate is between those of drugs A and C. Drug A reaches the highest peak plasma concentration, as all of the drug is absorbed before significant elimination can take place. Drug C is absorbed slowly and never achieves a high plasma concentration, but it persists in the plasma for longer than drugs A or B because absorption continues during the elimination phase. It should be noted that the hypothetical drugs A, B, and C could all be the same drug administered by three different routes. For example, curve A could represent intravenous glucocorticoid administration, curve B could be a depot intramuscular injection, and curve C could be an ultraslow-release subcutaneous formulation of the same drug.

## Mucous Membrane

Administration of drugs across mucous membranes can potentially provide rapid absorption, low incidence of infection, convenience of administration, and avoidance of harsh environments and first-pass metabolism. Sublingual, ocular, pulmonary, nasal, rectal, urinary, and reproductive tract epithelia have all been used to deliver drugs in the form of liquid drops, rapidly dissolving tablets, aerosols, and suppositories (among other dosage forms). The mucous membranes are highly vascular, permitting the drug to enter the systemic circulation rapidly and to reach its target organ with minimal delay. Drugs may also be administered directly into the target organ, resulting in virtually instantaneous onset of action. This is advantageous in such critical conditions as acute asthma, where such drugs as β-adrenergic agonists are administered via aerosol directly into the airways.

## Transdermal

A limited set of drugs has sufficiently high lipophilicity that passive diffusion across the skin provides a viable route of administration. Transcutaneously administered drugs are absorbed from the skin and subcutaneous tissues directly into the bloodstream. This route of administration is ideal for a drug that must be slowly and continuously administered over extended periods. There is no associated risk of infection, and drug administration is simple and convenient. The success of transdermal nicotine, estrogen, and scopolamine patches demonstrates the potential utility of this route of administration.

# LOCAL FACTORS AFFECTING ABSORPTION

The rate and extent of absorption of a drug are affected by several factors that are specific to the treatment situation. In general, a higher and/or more rapidly administered dose results in a greater increase in local drug concentration (Fig. 3-4). This increases the tendency for the drug to diffuse across membranes or into the bloodstream, which then causes the local drug concentration to decrease. Thus, factors that increase the rate of distribution of the drug away from the site of administration decrease the likelihood that the concentration of the drug will reach equilibrium across a biological membrane. Regional blood flow has the greatest effect in this regard; a highly perfused region rapidly removes a drug that crosses into that compartment. This effect maintains the drug concentration at a low level in the compartment, allowing the driving force for entry of a new drug into the compartment to remain high (see Equation 3-1). Volatile general anesthetics, for example, are administered via inhalation. The lungs are highly perfused, and the anesthetic is removed rapidly from the lungs into the circulation. Because blood moves through the lung rapidly, an increased concentration of anesthetic does not develop in the local circulation, and there is little diffusional force to oppose the entry of anesthetic into the bloodstream. Anesthetic will, therefore, enter unopposed until the bloodstream compartment starts to become saturated (see Chapter 14, General Anesthetic Pharmacology, for more details). A similar trend is seen in patients of greater mass, who have both an increased surface area across which diffusion can occur and a larger tissue volume in which the drug can be diluted. Both of these factors increase the tendency for a drug to be absorbed by the patient.

**TABLE 3-3** Drug Distribution to Different Body Compartments

| COMPARTMENT | EXAMPLES |
|---|---|
| Total body water | Small water-soluble molecules (e.g., ethanol) |
| Extracellular water | Larger water-soluble molecules (e.g., mannitol) |
| Blood plasma | Strongly plasma protein-bound molecules, very large molecules, highly charged molecules (e.g., heparin) |
| Fat | Highly lipid-soluble molecules (e.g., diazepam) |
| Bone | Certain ions (e.g., fluoride) |

## DISTRIBUTION

Although absorption of a drug is a prerequisite for establishing adequate plasma drug levels, the drug must also reach its target organ(s) in therapeutic concentrations to have the desired effect on a pathophysiologic process. Drug distribution is achieved primarily through the circulatory system; a minor component is contributed by the lymphatic system. Once a drug has been absorbed into the systemic circulation, it is then capable of reaching any target organ (with the possible exception of the brain and testes). The concentration of drug in the plasma is often used to determine therapeutic drug levels, because the amount of drug that is actually taken up by the target organ is difficult to measure. In some cases, the plasma concentration of a drug may represent a relatively poor measure of the actual tissue concentration. In most cases, however, the effect of the drug in the tissue correlates well with the plasma drug concentration.

Organs and tissues vary widely in their capacity to take up different drugs and in the proportion of systemic blood flow that these tissues receive. The forces governing the distribution of a drug among the various tissues and compartments (Table 3-3) greatly affect the concentration of the drug in the plasma. Blood flow also varies markedly among the different organ systems, with the liver and kidneys usually receiving the greatest flow (Table 3-4). These kinetic factors determine the amount of drug that must be administered to achieve the desired concentration of drug within the vascular compartment. The ability of nonvascular tissues and of plasma proteins to take up and/or bind the drug adds complexity to dosing regimens and must be accounted for if desired drug levels are to be reached.

## VOLUME OF DISTRIBUTION

The **volume of distribution** of a drug ($V_d$) represents the fluid volume that would be required to contain the total amount of absorbed drug in the body at a uniform concentration equivalent to that in the plasma at a steady state.

$$V_d = \frac{\text{Dose}}{[\text{Drug}]_{\text{plasma}}} \qquad \text{Equation 3-4}$$

The extent to which a drug is taken up by the body as a whole is greater when the drug is highly distributed among body tissues. Thus, the volume of distribution is relatively low for drugs that are retained primarily within the vascular compartment and relatively high for drugs that are highly distributed into muscle, adipose, and other nonvascular compartments. In fact, for very highly distributed drugs, the volume of distribution is often much greater than the volume of total body water, reflecting the low concentration of drug in the vascular compartment at steady state. For example, the volumes of distribution for drugs such as digoxin and labetalol are in the hundreds of liters for a typical 70-kg patient; this demonstrates that the volume of distribution is an extrapolated volume based on the concentration of drug in the plasma, rather than a physical volume. This concept is quantified in Equation 3-4.

The capacity of the bloodstream and the various body organs and tissues to take up and retain a drug depends on both the volume (mass) of the tissue and the density of specific and nonspecific binding sites for the drug within that tissue. A drug that is taken up in large quantities by such body tissues as adipose and muscle will be largely removed from the circulation at steady state. In most cases, these tissues must be saturated before plasma levels of such drugs can increase sufficiently to affect the drug's target organ. Thus, for drugs of equal potency, the drug that is more highly distributed among

**TABLE 3-4** Total and Weight-Normalized Tissue Blood Flow in an Adult

| ORGAN PERFUSED | BLOOD FLOW (mL/min) | ORGAN MASS (kg) | NORMALIZED BLOOD FLOW (mL/min/kg) |
|---|---|---|---|
| Liver | 1,700 | 2.5 | 680 |
| Kidney | 1,000 | 0.3 | 3,333 |
| CNS | 800 | 1.3 | 615 |
| Myocardium | 250 | 0.3 | 833 |
| Fat | 250 | 10.0 | 25 |
| Other (muscle, etc.) | 1,400 | 55.6 | 25 |
| Total | 5,400 | 70.0 | |

body tissues generally requires a higher initial dose to establish a therapeutic plasma concentration than does the drug that is less highly distributed.

## Plasma Protein Binding

The capacity of muscle and adipose tissue to bind a drug increases the tendency of the drug to diffuse from the bloodstream into nonvascular compartments, but this tendency can be counteracted to some extent by plasma protein binding of the drug. Albumin is the most abundant plasma protein (its concentration is about 4 g/dL) and is the protein that is most responsible for drug binding. Many drugs bind with low affinity to albumin through both hydrophobic and electrostatic forces. Plasma protein binding tends to reduce the availability of a drug for diffusion or transport into the drug's target organ because, in general, only the free or unbound form of the drug is capable of diffusion across membranes (Fig. 3-5). Plasma protein binding may also reduce the transport of drugs into such nonvascular compartments as adipose tissue and muscle. Because a highly protein-bound drug tends to remain within the vasculature, such a drug often has a relatively low volume of distribution (typically, 7–8 liters for a 70-kg person).

Theoretically, plasma protein binding could be important as a mechanism for some drug–drug interactions. Coadministration of two or more drugs, each of which is highly bound to plasma protein, could result in a higher-than-expected plasma concentration of the free form of either or both drugs. This would occur because the coadministered drugs compete for the same binding sites on plasma proteins. The increased free drug concentration could have the potential to cause increased therapeutic and/or toxic side effects of the drug. In such cases, one could imagine that the dosing regimen of one or both of the drugs would be adjusted so as to bring the free drug concentration back into the therapeutic range. In practice, however, it has been difficult to demonstrate any clinically significant drug–drug interactions caused by competition between the two drugs' binding to plasma protein. This somewhat surprising result may be attributable to the increased clearance of the free drugs as they are displaced from their plasma protein binding sites (see below).

## MODELING THE KINETICS AND THERMODYNAMICS OF DRUG DISTRIBUTION

Most drugs are distributed rapidly from the systemic circulation (intravascular compartment) to other compartments in the body. This **distribution phase** results in a sharp decrease in the plasma drug concentration shortly after intravenous administration of a drug bolus. Even after the drug equilibrates among its tissue reservoirs, the plasma drug concentration continues to decline because of drug elimination from the body. The rate of decline of plasma drug concentration during the elimination phase is slower than that during the distribution phase, because drug in the tissue reservoirs is in equilibrium with that in the intravascular compartment, and, therefore, drug can diffuse from the tissues back into the blood (Figs. 3-6, 3-7).

The tendency for a drug to be taken up by adipose and muscle tissue during the distribution phase results in a set of

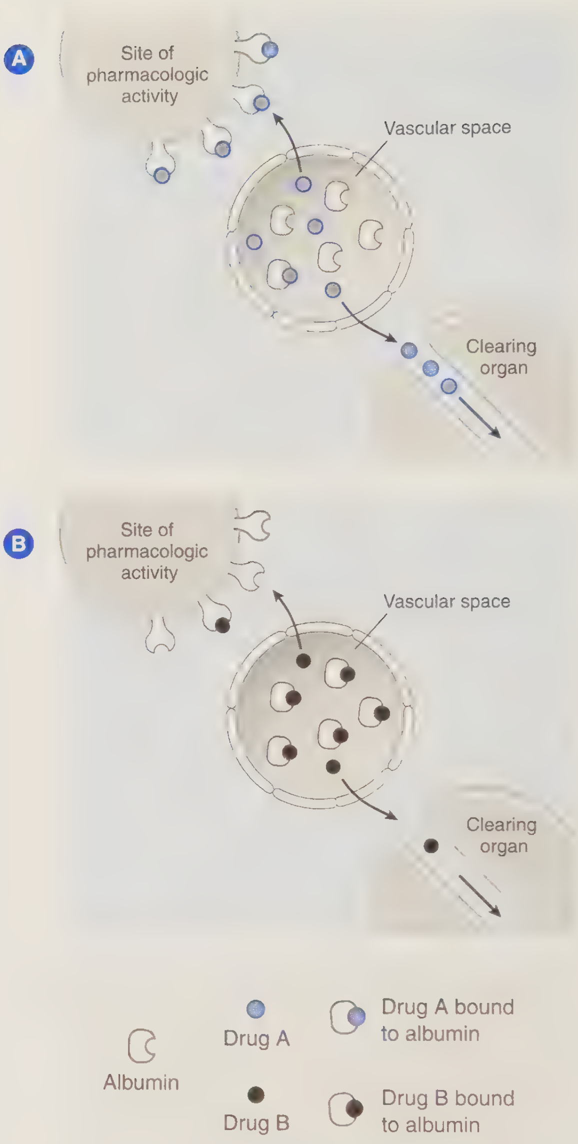


**Figure 3-5. Protein Binding and Drug Trapping.** A drug that is bound to albumin or other plasma proteins cannot diffuse from the vascular space into surrounding tissue. **A.** Drugs that do not bind plasma protein appreciably (shown here as Drug A) diffuse readily into tissues. This results in both a high level of binding to the site of pharmacologic activity (usually receptors) and a high rate of elimination (represented by flux through a clearing organ). Examples of such drugs include acetaminophen, acyclovir, nicotine, and ranitidine. **B.** However, if a drug exhibits high levels of binding to plasma protein (shown here as drug B), a higher total plasma drug concentration would be required to ensure an adequate concentration of free (unbound) drug in the circulation. Otherwise, little of the drug diffuses into the extravascular space, and only a small percentage of the receptors are occupied. Examples of such drugs include amiodarone, fluoxetine, naproxen, and warfarin. It should be emphasized that plasma protein binding is only one of many variables that determine drug distribution. Drug molecule size, lipophilicity, and rate of metabolism are other important parameters that must be taken into account when considering the pharmacokinetics of a particular drug.

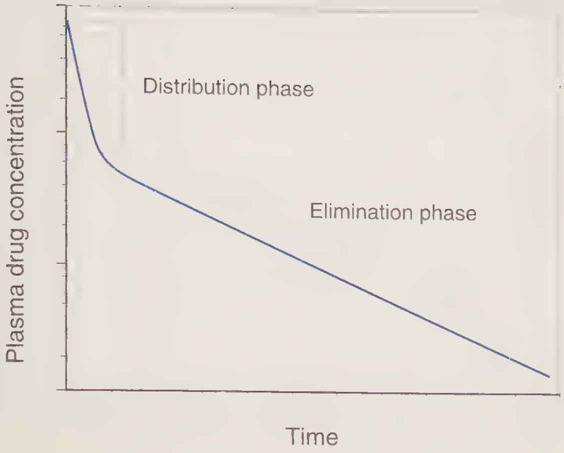


**Figure 3-6. Drug Distribution and Elimination Following Intravenous Administration.** Immediately following intravenous administration of a drug, the plasma drug concentration declines rapidly as the drug distributes from the vascular compartment to other body compartments. This rapid decline is followed by a slower decline as the drug is metabolized and excreted from the body. Both drug distribution and elimination display first-order kinetics, as demonstrated by linear kinetics on a semilogarithmic plot.

dynamic equilibria among drug concentrations in the various body compartments. As shown in Figure 3-8, the rapid decline of plasma drug concentration after administration of an intravenous bolus of drug can be approximated more accurately by using a four-compartment model consisting of the blood and vessel-rich, muscle-rich, and adipose-rich tissues. The vessel-rich group is the first extravascular compartment in which the concentration of the drug increases, because the high blood flow received by this group *kinetically* favors drug entry into this compartment. However, the muscle-rich group and fat group often have a higher *capacity* for taking up drug than the vessel-rich group. Because the fat group often has the highest capacity to take up drug and the lowest blood flow, the fat group can take up a greater amount of drug than the other groups, but at a slower rate.

The capacity of a compartment for drug and the rate of blood flow to the compartment also affect the rate at which drugs exit from that compartment. Drugs tend to exit from the vessel-rich group first, followed by the muscle group and then the fat group. Most drugs are taken up to greater or lesser extents by all of these tissues, creating a complex and dynamic pattern of changing blood concentrations over time that is specific for each particular drug. This pattern may also be patient-specific, depending on such factors as the size, age, and fitness level of a patient. For example, an older patient typically has less skeletal muscle mass than does a younger patient, decreasing the contribution of muscle uptake to changes in the plasma concentration of a drug. An opposite effect may be seen in an elite athlete, who would be expected to have both larger muscle mass and greater proportional muscle blood flow. As a final example, an obese person would be expected to exhibit greater capacity for drug uptake into adipose tissue.

## METABOLISM

A number of organs are capable of metabolizing drugs to some extent, using enzymatic reactions that are elucidated further in Chapter 4, Drug Metabolism. Thus, the kidneys, gastrointestinal tract, lungs, skin, and other organs all contribute to systemic drug metabolism. The liver contains the greatest diversity and quantity of metabolic enzymes, however, and the majority of drug metabolism occurs there. The ability of the liver to modify drugs depends on the amount of drug that enters into hepatocytes. Highly lipophilic drugs can generally enter cells (including liver cells) more readily. As a result, the liver preferentially metabolizes hydrophobic drugs. The liver is able to modify a variety of substituents on drugs chemically, thereby either rendering the drugs inactive or facilitating their elimination. These modifications are collectively referred to as **biotransformation.** Biotransformation reactions are classified into two types, termed **Phase I** and **Phase II** processes.

### PHASE I

Phase I reactions modify the chemical structure of a drug through oxidation, reduction, or hydrolysis. The liver has enzymes that facilitate each of these reactions. The most commonly used pathway, the microsomal cytochrome P450 system, mediates a large number of oxidative reactions. One common oxidative reaction involves the addition of a hydroxyl group to the drug. It should also be noted that some drugs are administered in inactive **(prodrug)** form, so that they can be altered metabolically by Phase I reactions to the active (drug) form in the liver. This prodrug strategy can be used to facilitate oral bioavailability, decrease gastrointestinal toxicity, and/or prolong the elimination half-life of a drug.

### PHASE II

Phase II reactions conjugate a drug to large polar molecules in order to inactivate the drug or, more commonly, to enhance the drug's solubility and excretion rate in the urine or bile. Occasionally, addition of these groups can result in the metabolic activation of prodrugs. The most commonly added groups include glucuronate, sulfate, glutathione, and acetate.

As described in more detail in the next chapter, the effects of Phase I and Phase II reactions on a particular drug also depend on the presence of other drugs that are being taken concomitantly by the patient. Certain classes of drugs, such as barbiturates, are powerful inducers of enzymes that mediate Phase I reactions; other drugs are capable of inhibiting these enzymes. An understanding of these drug–drug interactions is an essential prerequisite to the appropriate dosing of drug combinations.

## EXCRETION

Phase I and II biotransformation reactions serve to enhance the hydrophilicity of a hydrophobic drug and its metabolites, enabling such drugs to be excreted along a final common pathway with drugs that are intrinsically hydrophilic. Most

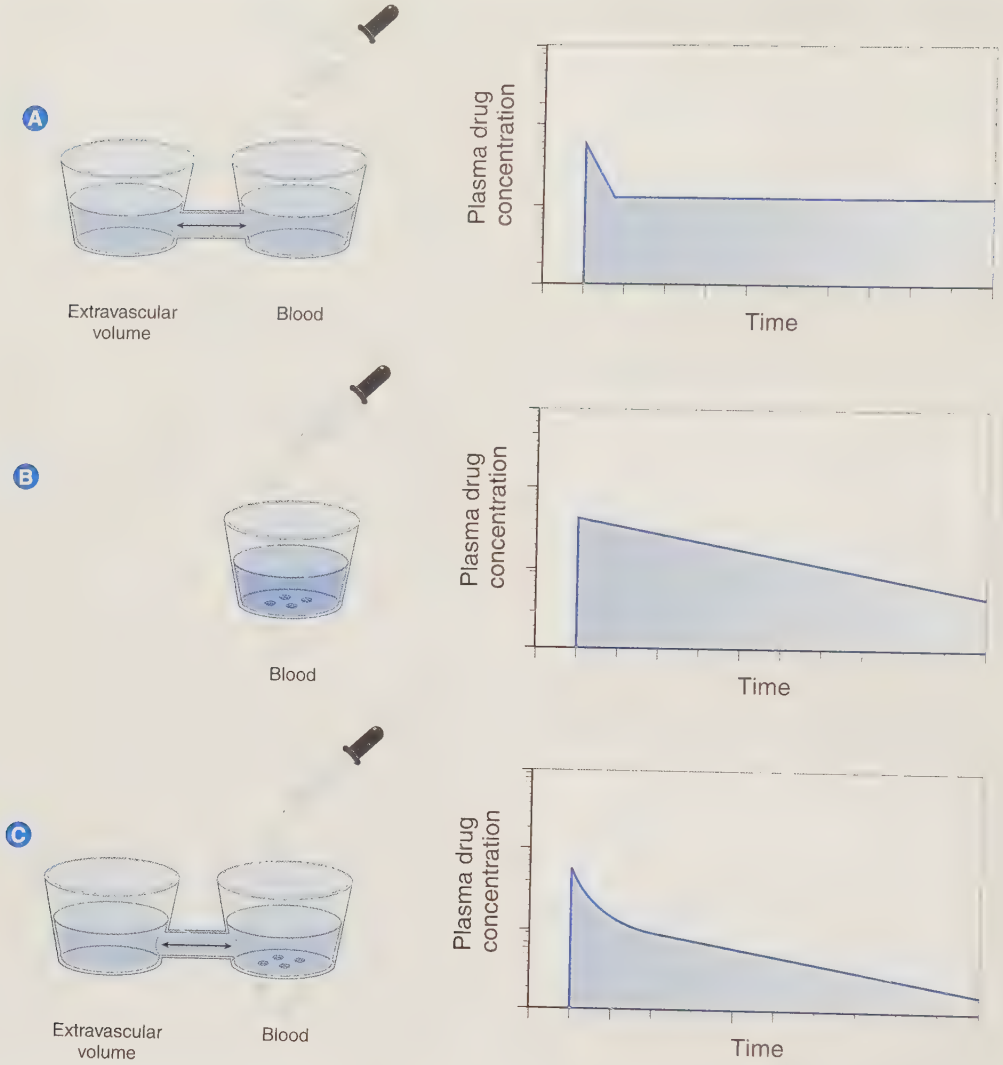

A
Extravascular volume
Blood
Plasma drug concentration
Time
B
Blood
Plasma drug concentration
Time
C
Extravascular volume
Blood
Plasma drug concentration
Time

**Figure 3-7. Schematic Model of Drug Distribution and Elimination.** A two-compartment pharmacokinetic model can be used to describe drug distribution and elimination following administration of a single intravenous dose. The drug concentration rises rapidly as the drug is added to the first compartment. In the absence of elimination, the initial rise in drug concentration is followed by a rapid decline to a new plateau as the drug equilibrates (distributes) between the two compartments **(A)**. If the distribution of the drug is confined to the blood volume, then the plasma drug concentration declines more slowly as the drug is eliminated from the body **(B)**. In both cases, as the concentration of drug in the plasma decreases, the forces driving drug distribution **(A)** and elimination **(B)** decrease, and the absolute amount of drug distributed or eliminated per unit time decreases. Therefore, the kinetics of both distribution and elimination appear as straight lines on a semilogarithmic plot; this is the definition of first-order kinetics. Note that the half-time for drug elimination is generally longer than the half-time for drug distribution. When drug distribution and elimination are occurring simultaneously, the decline of plasma drug concentration with time is represented by the sum of the two processes **(C)**. Note that the curve in **C** is the sum of the two first-order processes shown in **A** and **B**.

drugs and drug metabolites are removed to the exterior of the body through renal and biliary excretion. Renal excretion provides the most common mechanism of drug excretion; this form of excretion relies on the hydrophilic character of a drug or metabolite. Only a relatively small number of drugs are excreted primarily in the bile. Many orally administered drugs are incompletely absorbed from the upper gastrointestinal tract, and residual drug is eliminated by fecal excretion. Otherwise, drugs can be excreted in minor quantities through respiratory and dermal routes.

## RENAL EXCRETION

Renal blood flow comprises about 25% of total systemic blood flow, ensuring that the kidneys are continuously exposed to any drug found in the blood. The rate of drug elimination through the kidneys depends on the balance of drug filtration, secretion, and reabsorption rates (Fig. 3-9). The afferent arteriole introduces both free (unbound) drug and plasma protein-bound drug into the glomerulus. Typically, however, only the free drug form is filtered into the renal

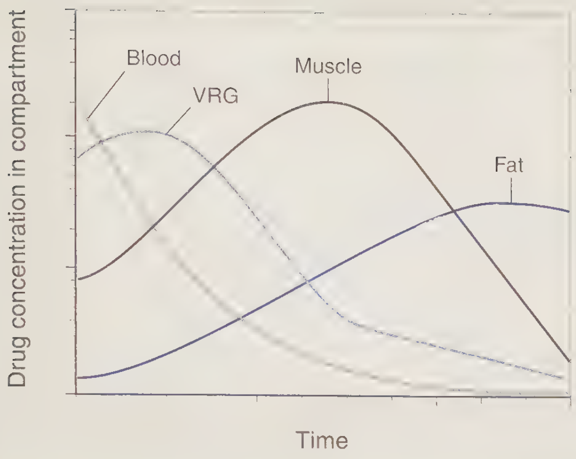


**Figure 3-8. Four-Compartment Model of Drug Distribution.** Following administration of an intravenous bolus, the drug is delivered to various tissues via the bloodstream. Drug concentration is initially highest in the vascular compartment (blood), but subsequently falls rapidly as the drug is distributed to the different tissue compartments. The most vessel-rich tissues (i.e., the tissues that are supplied by the highest fraction of the cardiac output) are generally the first to accumulate drug. However, the tissue compartments also vary in their capacity for taking up drug. Because the mass of the muscle group is larger than that of the vessel-rich group (VRG), the muscle group has a larger uptake capacity. Because the muscles are less well perfused than the vessel-rich group, this effect is manifested only after the drug has begun to distribute to the vessel-rich group. The most poorly perfused group is the fat group, but this group has the highest capacity to accumulate drug. The peak level of drug in the fat group does not reach that in the muscle-rich group, because a significant amount of drug has been eliminated by metabolism or excretion before the fat group begins to accumulate drug. Upon further drug elimination from the body, the reverse pattern is seen—the drug leaves first from the vessel-rich group and then from the muscle and fat groups, respectively.

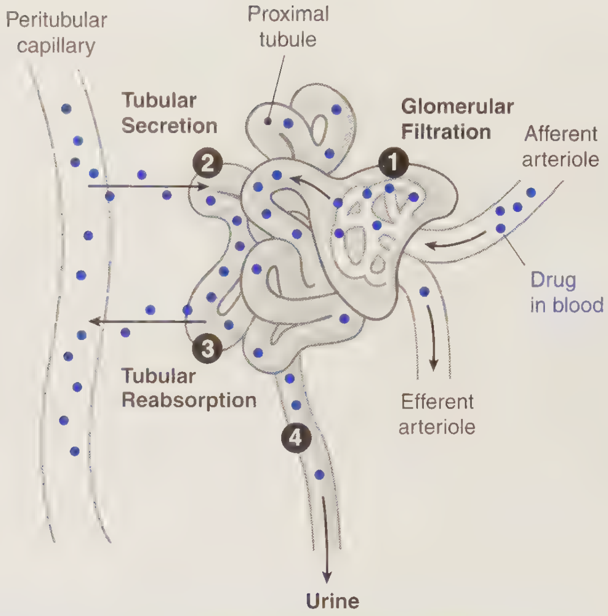


**Figure 3-9. Drug Filtration, Secretion, and Reabsorption in the Kidney.** Drugs may be filtered at the renal glomerulus **(1)**, secreted into the proximal tubule **(2)**, reabsorbed from the tubular lumen and transported back into the blood **(3)**, and excreted in the urine **(4)**. Therefore, the relative balance of filtration, secretion, and reabsorption rates determines the kinetics of drug elimination by the kidney. Enhancing blood flow, increasing glomerular filtration rate, and decreasing plasma protein binding all cause a drug to be more rapidly excreted, because all these changes result in increased delivery of drug to the glomerulus. Some drugs, such as penicillin, are actively secreted at the proximal tubule. Although reabsorption can decrease the elimination rate of a drug, many drugs exhibit pH trapping in the distal tubule and are, therefore, efficiently excreted in the urine. For drugs that are dependent on the kidney for elimination, compromised renal function can result in higher plasma drug concentrations, and the dose and frequency of drug administration must be altered accordingly.

tubule. Therefore, renal blood flow, glomerular filtration rate, and drug binding to plasma protein all affect the amount of drug that enters the tubule at the glomerulus. Enhancing blood flow, increasing glomerular filtration rate, and decreasing plasma protein binding all cause a drug to be excreted more rapidly. Renal excretion plays a role in the clearance of many drugs, and is usually the primary route of excretion of such drugs as vancomycin, atenolol, and ampicillin. Drugs can accumulate to toxic levels in patients with compromised renal function; this is often an important consideration in elderly patients.

Urinary drug concentration rises in the proximal tubule because of passive diffusion of uncharged drug molecules, facilitated diffusion of charged or uncharged molecules, and active secretion of anionic and cationic molecules from the blood into the urinary space. The secretory mechanisms are generally not specific for the drugs; rather, drug secretion takes advantage of molecular similarities between the drug and such naturally occurring substances as organic acids and bases. The extent of protein binding has a relatively small effect on drug secretion into the proximal tubule, indicating that there is usually a rapid equilibrium between protein-bound and unbound forms of the drug in the blood.

The urinary concentration of drug may fall as the drug is reabsorbed in the proximal and distal tubule. Reabsorption is limited primarily by pH trapping, as described above. The renal tubular fluid is typically acidic in and beyond the proximal tubule, which tends to favor trapping of the ionic form of weak bases. Because this region of the tubule contains transporter proteins that are different from those in preceding segments of the nephron, ionic drug forms resist facilitated diffusional reabsorption, and their excretion is thereby enhanced. Drug reabsorption in the tubule can be enhanced or inhibited by chemical adjustment of the urinary pH. Changing the rate of urine flow through the tubules can also modify the rate of drug reabsorption. An increased rate of urine output tends to dilute the drug concentration in the tubule and to decrease the amount of time during which facilitated diffusion can occur; both of these effects tend to decrease drug reabsorption. For example, aspirin is a weak acid that is excreted renally. Aspirin overdose is treated by administering sodium bicarbonate to alkalinize the urine (and thus trap aspirin in the tubule) and by increasing the urine flow rate (and thus diluting the tubular concentration of aspirin). Both of these clinical maneuvers result in faster elimination of the drug.

### BILIARY EXCRETION

Drug reabsorption also plays an important role in biliary excretion. Drugs that are secreted by the liver into the bile duct enter the gastrointestinal tract in the duodenum. Such drugs must, therefore, pass through the length of the small and large intestine before being excreted. In many cases, these drugs undergo enterohepatic circulation, in which they are reabsorbed in the small intestine and subsequently retained in the portal and then the systemic circulation. Such drugs as steroids, digoxin, and some cancer chemotherapeutic agents are largely excreted in the bile.

## CLINICAL APPLICATIONS OF PHARMACOKINETICS

The dynamic interactions among drug absorption, distribution, metabolism, and excretion determine the plasma concentration of a drug and dictate the ability of the drug to reach its target organ in an effective concentration. Often, the desired duration of drug therapy exceeds that achievable by a single dose, and multiple doses are needed to provide a relatively constant plasma concentration of drug within the limits of efficacy and toxicity. The results of clinical trials of drugs under development, as well as clinical experience using FDA-approved drugs, suggest target plasma levels for a drug in the average patient. Pharmacokinetic and other differences among patients must also be considered, however, in designing a dosing regimen for a drug or drug combination in the individual patient.

### CLEARANCE

The clearance of a drug is the pharmacokinetic parameter that most significantly limits the time course of action of the drug at its molecular, cellular, and organ targets. Clearance can be conceptualized in two complementary ways. First, it is defined as the rate of elimination of the drug from the body relative to the concentration of the drug in plasma. Alternatively, clearance is the rate at which plasma would have to be cleared of the drug to account for the observed kinetics of change of the total amount of drug in the body, assuming that all the drug in the body is present at the same concentration as that in the plasma. Therefore, clearance is expressed in units of volume/time, as follows:

$$\text{Clearance} = \frac{\text{Metabolism} + \text{Excretion}}{[\text{Drug}]_{\text{plasma}}} \qquad \text{Equation 3-5}$$

where metabolism and excretion are expressed as rates (amount/time).

Although metabolism and excretion are distinct physiologic processes, the pharmacologic endpoint is equivalent—a reduction in circulating levels of active drug. As such, metabolism and excretion are often referred to collectively as **clearance** mechanisms, and the principles of clearance can be applied to both:

$$\text{Clearance}_{\text{total}} = \text{Clearance}_{\text{renal}} + \text{Clearance}_{\text{hepatic}} + \text{Clearance}_{\text{Other}} \qquad \text{Equation 3-6}$$

### Metabolism and Excretion Kinetics

The rate of drug metabolism and excretion by an organ is limited by the rate of flow of blood to that organ. The majority of drugs commonly prescribed demonstrate **first-order kinetics** when used in standard therapeutic doses; that is, the amount of drug that is metabolized or excreted in a given unit of time is directly proportional to the concentration of drug in the systemic circulation at that time. Because the clearance mechanisms for most drugs are not saturated under ordinary circumstances, increases in plasma drug concentration are matched by increases in the rate of drug metabolism and excretion (see Equation 3-5). The first-order elimination rate (where elimination includes both metabolism and excretion) follows Michaelis–Menten kinetics:

$$E = \frac{V_{\max} \times C}{K_m + C} \qquad \text{Equation 3-7}$$

where $V_{\max}$ is the maximum rate of drug elimination, $K_m$ is the drug concentration at which the rate of elimination is $\frac{1}{2}V_{\max}$, $C$ is the concentration of drug in the plasma, and $E$ is the elimination rate (Fig. 3-10).

A small number of clinically used drugs (e.g., phenytoin) demonstrate **saturation kinetics,** in which the clearance mechanisms become saturated at or near the therapeutic concentration of the drug. Once saturation occurs, the clearance

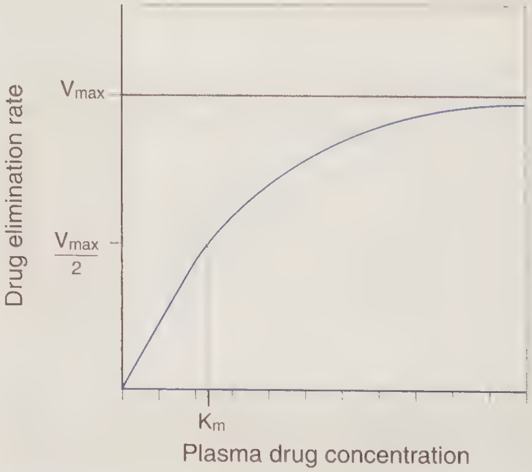


**Figure 3-10. Michaelis-Menten Kinetics.** Drug elimination typically follows Michaelis–Menten (first-order) kinetics. The rate of elimination increases as the plasma drug concentration increases, until the elimination mechanisms become saturated and reach a maximal elimination rate ($V_{\max}$) at high plasma concentrations. $K_m$ is the drug concentration at which the drug elimination rate is 1/2 $V_{\max}$.

rate fails to increase with increasing plasma drug concentrations. Instead, the clearance rate remains constant (a characteristic of **zero-order** rather than first-order kinetics) despite increasing plasma drug levels. This can result in dangerously elevated plasma concentrations of the drug and in toxic (or even lethal) side effects.

The extent to which an organ contributes to the clearance of drug is quantified by its **extraction ratio,** which compares the drug levels in plasma immediately before entering and just after exiting the organ:

$$\text{Extraction} = \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{Equation 3-8}$$

where $C$ = concentration. An organ that contributes significantly to drug clearance is expected to have a higher extraction ratio (closer to one) than does an organ that does not participate significantly in drug clearance (closer to zero).

## HALF-LIFE

By decreasing the concentration of active drug in the bloodstream, drug metabolism and excretion shorten the time during which a drug is capable of acting on a target organ. The **elimination half-life** of a drug, defined as the amount of time over which the drug concentration in the plasma decreases to one-half of its original value, is a useful parameter that allows the clinician to estimate the amount of time over which the concentration of a drug will remain in the therapeutic range. There are many potentially confounding factors in any clinical scenario, and it is most useful to consider here the simplest of cases. Because most drugs are eliminated by first-order kinetics, the body can often be modeled as a single compartment with a volume that is equivalent to the volume of distribution. In this model, the drug half-life $t_{1/2}$ depends only on the volume of distribution and clearance of the drug:

$$t_{1/2} = \frac{0.693 \times V_d}{\text{Clearance}} \qquad \text{Equation 3-9}$$

where $V_d$ is the volume of distribution, and 0.693 is an approximation of ln 2.

Thus, all of the factors outlined above that affect the volume of distribution and clearance of a drug also affect the half-life of the drug. A decrease in drug clearance or increase in volume of distribution tends to prolong the drug half-life and, thereby, to enhance the effect of the drug on the target organ. The half-life must be carefully considered in designing any dosing regimen, as the effects from a drug with a long half-life may last for a number of days. For example, the half-life of chloroquine is more than 1 week.

### Factors Altering Half-Life

Physiologic and pathologic changes in the volume of distribution must be considered when determining the appropriate drug dose and dosing interval (Table 3-5). As patients age, their skeletal muscle mass decreases, which could decrease the volume of distribution. In contrast, an obese person shows an increase in the amount of drug uptake by the fat group. A drug that distributes into the fat group must be given in a higher dose in order to reach therapeutic plasma drug levels. As a third example, if drug dosage is based on body weight but the fat group does not take up the drug, then potentially toxic drug levels could be reached in an obese individual. Finally, some drugs may partition preferentially into such pathologic fluid spaces as ascites or a pleural effusion, causing long-term toxicity if the drug dosage is not adjusted accordingly.

Physiologic and pathologic processes may also affect drug clearance. For example, the cytochrome P450 enzymes responsible for drug biotransformation in the liver can be induced, increasing the rate of drug inactivation, or inhibited, decreasing the rate of drug inactivation. The P450 system can be induced by some drugs (such as glucocorticoids and carbamazepine) and inhibited by others (such as quinidine and cimetidine; see Box 4-1). Organ failure is another critical factor in determining appropriate drug regimens. Liver failure may both alter liver enzyme function and decrease biliary excretion. Decreased cardiac output reduces the amount of blood that reaches clearance organs. Renal failure decreases drug excretion because of decreased drug filtration and secretion into the renal tubules (see Box 3-1). In summary, hepatic, cardiac, and renal failure can each lead to a decreased ability to inactivate or eliminate a drug, and, thereby, increase the elimination half-life of the drug.

**TABLE 3-5** Factors Affecting Drug Half-life

| FACTORS AFFECTING HALF-LIFE | MOST COMMON EFFECT ON HALF-LIFE |
|---|---|
| **Effects on Drug Distribution** | |
| Aging (decreased muscle mass → decreased distribution) | Decreased |
| Obesity (increased adipose mass → increased distribution) | Increased |
| Pathologic fluid (increased distribution) | Increased |
| **Effects on Drug Clearance** | |
| Cytochrome P450 induction (increased metabolism) | Decreased |
| Cytochrome P450 inhibition (decreased metabolism) | Increased |
| Hepatic failure (decreased clearance) | Increased |
| Cardiac failure (decreased clearance) | Increased |
| Renal failure (decreased clearance) | Increased |

UNIVERSITY COLLEGE Library CORK

### BOX 3-1. Application to Therapeutic Decision-Making: Drugs in Renal Failure (by Vivian Gonzalez Lefebre and Robert H. Rubin)

Many drugs are excreted by the kidneys, and the decreased creatinine clearance that accompanies chronic renal failure often necessitates dosage adjustment or use of a different drug. Consider the following clinical scenario.

Mr. Roberts is a 59-year-old man with diabetes mellitus, hypertension, and chronic renal failure (creatinine clearance < 10 mL/min). He has required hemodialysis for 5 years. One night he is admitted to the hospital with fever and hypotension. The presumed source of infection is the central venous catheter used for hemodialysis. Blood cultures are obtained from both the catheter and a peripheral site. The gram stain of the catheter tip is remarkable for gram-positive cocci, and empiric treatment is started with vancomycin and gentamicin. The culture eventually identifies methicillin-resistant *Staphylococcus aureus* (MRSA).

Renal disease can lead to many physiologic changes that affect the pharmacokinetics of a drug. For example, in patients with edema, pleural effusion, or ascites, there may be an increase in the volume of distribution of highly water-soluble or protein-bound drugs. The most concerning pharmacologic consequence of renal insufficiency, however, is its effect on drug clearance. Plasma concentrations of drugs, such as gentamicin and methotrexate, which have narrow therapeutic indices and are predominantly cleared by the kidneys, may reach sustained, toxic levels if administered at standard doses to a patient with renal failure. Therefore, the doses of these drugs must be reduced in proportion to the extent of renal compromise. Renal function is most commonly measured by creatinine clearance; using the plasma creatinine level to establish the presence of normal renal status can be misleading because the plasma creatinine may fall within the normal range in elderly or debilitated patients with mild to moderate renal failure as a result of diminished muscle mass. Assuming that such patients have normal renal function can cause serious overdoses and toxic drug accumulation.

Renal insufficiency may also alter the pharmacodynamics of some drugs. Potassium salts, potassium-sparing diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), and ACE inhibitors are more likely to cause hyperkalemia in patients with renal dysfunction. Thiazide diuretics tend to be ineffective in a patient whose glomerular filtration rate is less than 30 mL/min because these drugs must be secreted by the kidney to act at the luminal membrane of the renal tubules. Refer to Chapter 19, Pharmacology of Volume Regulation, for a review of the physiology and pharmacology of diuretic action.

How does Mr. Roberts's chronic renal failure affect the choice and dosing of a safe and effective antibiotic regimen for his infection? Infectious complications are a source of substantial morbidity and a common cause of death among dialysis patients. Gram-positive organisms, including *S. aureus,* are responsible for most catheter-related infections. (Because septicemia is a therapeutic emergency, however, empiric treatment should not be delayed while waiting for culture results. Therapy can be modified subsequently, once the culture and sensitivity results are known.) In this case, empiric treatment consists of vancomycin and gentamicin for broad-spectrum coverage of gram-positive and gram-negative organisms. Gentamicin, an aminoglycoside, is often used to treat infections caused by gram-negative bacilli. It is eliminated renally and is effectively removed by hemodialysis. Hence, it is usually administered immediately after a hemodialysis session. In the case of Mr. Roberts, gentamicin would be discontinued once the cultures reveal methicillin-resistant *S. aureus.* The tricyclic glycopeptide vancomycin is the antibiotic of choice for MRSA infections. Vancomycin is cleared by the kidneys but, unlike the small molecule gentamicin, is not removed by conventional hemodialysis. In individuals with normal renal function, the dosing interval for vancomycin is 12 hours. In severe renal failure, as in this case, therapeutic levels of the drug may persist for 7 days following a single intravenous dose, allowing outpatient treatment once the patient has become hemodynamically stable.

## THERAPEUTIC DOSING AND FREQUENCY

The basic principles of pharmacokinetics—absorption, distribution, metabolism, and excretion—each influence the design of an optimal dosing regimen for a drug. Absorption determines the potential route(s) of administration and helps to determine optimal drug dosage. A highly absorbed drug—as evidenced by a high bioavailability—generally requires a lower dosage than does a poorly absorbed drug. (It is important to note, however, that the most important determinant of drug dosage is the **potency** of the drug; see Chapter 2.) In contrast, a highly distributed drug—as evidenced by a higher volume of distribution—necessitates higher drug dosing. The elimination rate of a drug influences its half-life and, therefore, determines the frequency of dosing required to maintain therapeutic plasma drug levels.

In general, *therapeutic dosing of a drug seeks to maintain the peak (highest) plasma drug concentration below the toxic concentration, and the trough (lowest) drug concentration above the minimally effective level* (Fig. 3-11). This can be accomplished most efficiently using continuous drug delivery by the intravenous (continuous infusion), subcutaneous (continuous pump or implant), oral (sustained-release tablet), and other routes of administration, as described in more detail in Chapter 50, Drug Delivery Mechanisms. In many cases, however, the dosing regimen must also consider patient convenience and provider availability. Frequent small doses (usually oral) can be administered to achieve minimal variation in steady-state plasma drug concentration, but this strategy subjects the patient to the inconvenience of frequent drug administration. Less frequent dosing requires the use of higher doses and leads to greater fluctuations in peak and trough drug levels; this type of regimen is more convenient for the patient but also more likely to cause problems due to excessive (toxic) or insufficient (subtherapeutic) drug levels (Fig. 3-12). Optimum dosing regimens maintain the steady-state plasma drug

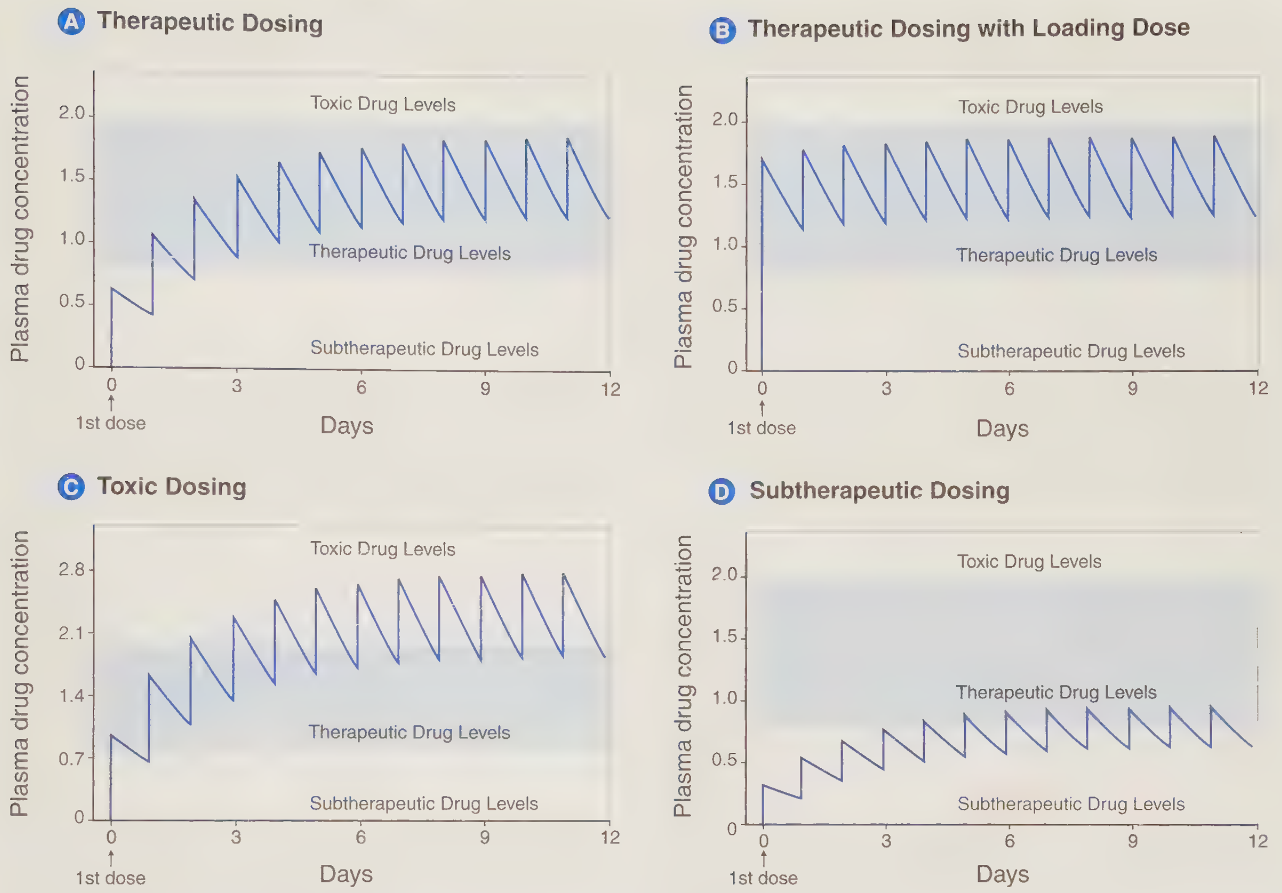


**Figure 3-11. Therapeutic, Subtherapeutic, and Toxic Drug Dosing.** From a clinical perspective, drug concentrations in plasma can be divided into subtherapeutic, therapeutic, and toxic drug levels. The goal of most drug-dosing regimens is to maintain the drug at concentrations above the subtherapeutic level but below the toxic level (referred to as the "therapeutic window"). **A.** The first several doses of a drug are typically subtherapeutic, as the drug equilibrates to its steady-state concentration (approximately four elimination half-times are required to achieve steady state). Appropriate drug dosing and dosing frequency result in steady-state drug levels that are therapeutic, and the maximal and minimal concentrations of the drug remain within the therapeutic window. **B.** If the initial (loading) dose is larger than the maintenance dose, the drug reaches therapeutic concentrations more rapidly. The magnitude of the loading dose is determined by the volume of distribution of the drug. **C.** Excessive maintenance dosing or dosing frequency results in drug accumulation and toxicity. **D.** Insufficient maintenance dosing or dosing frequency results in a subtherapeutic steady-state drug concentration. In all four panels, the drug is administered once daily, and the drug is assumed to distribute very rapidly to the various body compartments.

concentration within the therapeutic window. Because steady state is reached when the rate of drug input is equal to its output, the steady-state drug concentration is affected by drug bioavailability, clearance, dose, and dosing interval (the frequency of administration):

$$C_{\text{steady state}} = \frac{\text{Bioavailability} \times \text{Dose}}{\text{Interval}_{\text{dosing}} \times \text{Clearance}} \qquad \text{Equation 3-10}$$

where $C$ is the plasma concentration of the drug.

Immediately following the initiation of drug therapy, the rate of drug entry into the body ($k_{\text{in}}$) is much greater than the elimination rate ($k_{\text{out}}$ or $k_e$); therefore, the drug concentration in the bloodstream increases. As the plasma drug concentration increases, the rate of elimination also increases, because this rate is proportional to the plasma drug concentration. Steady state is reached when the two rates ($k_{\text{in}}$ and $k_{\text{out}}$) are equivalent. Because $k_{\text{in}}$ is a constant, *the approach to steady state is governed by* $k_e$, *the composite rate for all drug clearance mechanisms.* In multiple dosing regimens, drug levels accumulate following each successive dose, and the steady state is reached only when the amount of drug entering the system is equivalent to the amount being removed from it. Clinically, this principle must be remembered when the dosing regimen is altered, because at least four to five elimination half-lives must pass before the new steady state is reached.

The steady-state plasma concentration can also be altered by the addition of another drug to a patient's treatment regimen. In the case of Mr. Walsh, the addition of the drug trimethoprim-sulfamethoxazole inhibited the metabolism of warfarin, decreasing the clearance rate of the latter drug and causing its steady-state concentration to reach supratherapeutic levels. Assuming that Mr. Walsh's weight is approximately 70 kg, that he is taking 5 mg of warfarin every 24 hours, and

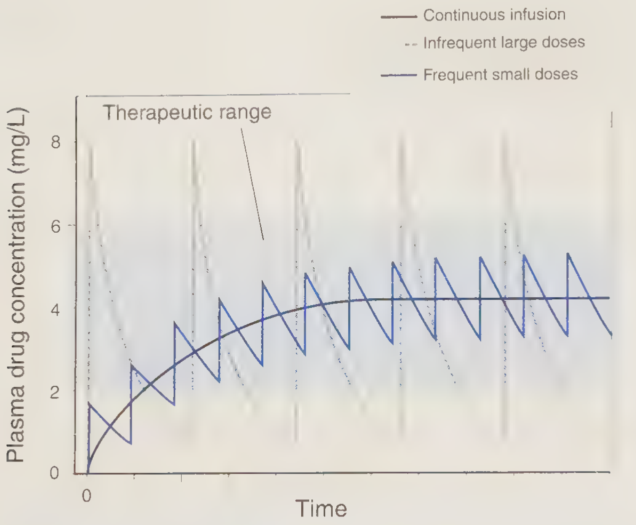


**Figure 3-12. Fluctuations in Steady-State Drug Concentration Depend on Dosing Frequency.** The same average steady-state plasma drug concentration can be reached using a variety of different drug doses and dosing intervals. In the example shown, the same total amount of a drug is administered by three different dosing regimens: continuous infusion; frequent small doses; and infrequent large doses. The smooth line represents the effect of a continuous infusion of drug. Less frequent dosing intervals require greater individual drug doses and result in larger fluctuations above and below the steady-state concentration. Note that all three dosing regimens have the same time-averaged plasma drug concentration at steady state (4 mg/L), but that the infrequent large dose regimen results in higher peaks and lower troughs above and below the target drug concentration. If these peaks and troughs fall above or below the boundaries of the therapeutic window, then the clinical outcome can be adversely affected. For this reason, frequent small doses are generally more efficacious and better tolerated than infrequent large doses. However, this concern must be balanced against the convenience of, and improved patient compliance with, occasional (e.g., once daily) dosing regimens.

that the bioavailability of warfarin is 0.93, then the initial steady-state plasma concentration of warfarin can be calculated as follows:

$$C_{\text{steady state}} = \frac{0.93 \times 5\ \text{mg}}{24\ \text{h} \times\ 0.192\ \ \text{L/h}} = 1.01\ \text{mg/L}$$

where the clearance value of 0.192 L/h is determined from the half-life and volume of distribution of warfarin (see Equations 3-9, 3-10). If the clearance of warfarin were decreased by the addition of trimethoprim-sulfamethoxazole, then the steady-state concentration of warfarin would increase and could potentially reach toxic levels.

## Loading Dose

Following the administration of a drug by any route, the plasma concentration of the drug initially increases. Distribution of drug from the vascular (blood) compartment to body tissues then causes the plasma drug concentration to decrease. The rate and extent of this decrease are significant for drugs with high volumes of distribution. If the administered dose of drug fails to take account of this volume of distribution, instead accounting only for the blood volume, then therapeutic drug levels will not be reached promptly. Initial (loading) doses of drug are often administered in order to compensate for drug distribution into the tissues. Such doses may be much higher than would be required if the drug were retained in the intravascular compartment. Loading doses may be used to achieve therapeutic levels of drug (i.e., levels at the desired steady-state concentration) with only one or two doses of drug:

$$\text{Dose}_{\text{loading}} = V_d \times C_{\text{steady state}} \qquad \text{Equation 3-11}$$

where $V_d$ is the volume of distribution, and $C$ is the desired steady-state plasma concentration of the drug.

In the absence of a loading dose, four to five elimination half-lives are required for a drug to reach equilibrium between tissue distribution and plasma concentration of the drug. Use of a loading dose circumvents this process by providing a sufficient amount of drug to attain an appropriate (therapeutic) drug concentration in the blood and tissues after only one or two doses of drug. For example, warfarin has a volume of distribution of 9.8 L in a 70-kg person. Assuming a desired steady-state plasma concentration of 1.01 mg/L, the appropriate loading dose of warfarin in Mr. Walsh can be calculated as:

$$\text{Dose}_{\text{loading}} = 9.8\ \text{L} \times 1.01\ \ \text{mg/L} = 9.90\ \text{mg}$$

## Maintenance Dose

Once the steady-state plasma drug concentration is achieved, and equilibrium is established between the drug concentra-

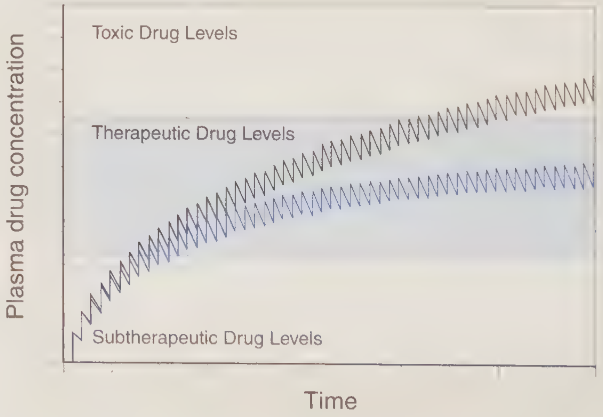


**Figure 3-13. Saturation Kinetics and Drug Toxicity.** Drug elimination typically follows first-order Michaelis–Menten kinetics, increasing as the plasma drug concentration increases. At optimal dosing, the steady-state plasma drug concentration remains within the therapeutic window (bottom curve). However, excessive drug dosing may saturate the body's capacity to eliminate the drug, for example, by overwhelming the hepatic cytochrome P450 enzyme system. In this case, the elimination rate of the drug does not increase with increasing plasma drug concentration (i.e., elimination follows zero-order rather than first-order kinetics). Continued administration of the drug results in drug accumulation, and the plasma drug concentration may reach toxic levels (top curve).

tions in the tissues and the plasma, subsequent doses must replace only the amount of drug that is lost through metabolism and excretion. The maintenance dose rate of a drug is dependent on the drug clearance, according to the principle that rate in = rate out at steady state:

$$\text{Dose}_{\text{maintenance}} = \text{Clearance} \times C_{\text{steady state}} \qquad \text{Equation 3-12}$$

Administration of a dose rate greater than this calculated maintenance dose rate would provide a drug input greater than the drug clearance, and the drug could accumulate to toxic levels within the tissues. In Mr. Walsh, the required maintenance dose for warfarin can be calculated as:

$$\begin{aligned}\text{Dose}_{\text{maintenance}} &= 0.192\ \text{L/h} \times 1.01\ \text{mg/L}\\ &= 0.194\ \text{mg/h} = 4.65\ \text{mg/day}\end{aligned}$$

The appropriate maintenance dose for Mr. Walsh is, therefore, 4.65 mg/day. Because warfarin is only 93% bioavailable, Mr. Walsh should take 5 mg/day to maintain an adequate steady-state plasma concentration. (Note also that, because warfarin has a low therapeutic index and toxic levels of the drug can lead to potentially fatal hemorrhage, the biologic activity of warfarin should be monitored carefully by periodic measurement of the prothrombin time.)

As noted above, the elimination of most drugs typically follows first-order kinetics, increasing as the concentration of drug in the plasma ($[D]_{\text{plasma}}$) increases. For some drugs, however, the body's capacity to eliminate the drug (e.g., through hepatic metabolism) may become saturated at therapeutic or only slightly supratherapeutic plasma drug concentrations. In these cases, the kinetics of drug elimination may change from first-order to zero-order, and the elimination rate of the drug does not increase with increasing values of $[D]_{\text{plasma}}$. Continued administration of the drug then results in rapid accumulation of drug in the plasma, and drug concentrations may reach toxic levels (Fig. 3-13).

## *Conclusion and Future Directions*

This chapter has provided an overview of the pharmacokinetic processes of absorption, distribution, metabolism, and excretion. An understanding of the factors that determine a drug's ability to act within a patient, and the changing nature of these factors over time, is vitally important to the safe and efficacious use of drug therapy. The key equations governing the relationships among dosing, clearance, and plasma drug concentration (Table 3-6) are important to consider when making therapeutic decisions about drug regimens.

**TABLE 3-6** Summary of Key Pharmacokinetic Relationships

| | | |
|---|---|---|
| Initial concentration | = | $\dfrac{\text{Loading dose}}{\text{Volume of distribution}}$ |
| Steady-state concentration | = | $\dfrac{\text{Fraction absorbed} \times \text{Maintenance dose}}{\text{Dosing interval} \times \text{Clearance}}$ |
| Elimination half-life | = | $\dfrac{0.693 \times \text{Volume of distribution}}{\text{Clearance}}$ |

At present, the clinical applicability of pharmacokinetics is generally based on drug effects observed in a population of individuals. There are nearly infinite major and minor variations among individuals, however, and these variations influence the effects of drug therapy. For example, clear differences in pharmacokinetics are present among persons of different age, gender, body mass, fitness level, ethnicity, and disease states. For some drugs, advances in therapeutic drug monitoring have enabled the real-time determination of plasma drug concentrations. A more extraordinary revolution in pharmacokinetics is offered by pharmacogenomics. Future drug therapy may involve the administration of drugs that have been engineered specifically for the patient who is receiving them. Knowledge of a patient's genomic makeup could enable drug therapies to exploit strengths and compensate for weaknesses in a host of patient-specific variables. This topic is discussed in detail in Chapter 52, Pharmacogenomics.

## *Suggested Readings*

Godin DV. Pharmacokinetics: Disposition and metabolism of drugs. In: Munson PL, ed. Principles of Pharmacology. New York: Chapman & Hall, 1995. (*A solid introductory text, this chapter illustrates the various aspects of pharmacokinetics with many examples of specific drugs.*)

Pratt WB, Taylor P, eds. Principles of Drug Action: The Basis of Pharmacology, 3rd Ed. New York: Churchill Livingstone, 1990, Chapters 3 and 4. (*This text is exhaustive in its treatment of pharmacokinetics and pharmacokinetic principles.*)

# 4

# Drug Metabolism

Janet F. McLaren, Joan J. Ryoo, and Christopher T. Walsh

**Introduction**
**Case**
**Sites of Drug Metabolism**
**Pathways of Drug Metabolism**
    Phase I Reactions
    Phase II Reactions
    Induction and Inhibition
    Active and Toxic Metabolites
**Individual Factors Affecting Drug Metabolism**
    Pharmacogenomics
    Age and Gender
    Diet and Environment
    Metabolic Drug Interactions
    Diseases Affecting Drug Metabolism
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Rarely does a compound that enters the body leave without some form of modification. The biologic transformations performed by the body can alter the compound to render it beneficial, harmful, or simply ineffective. The term **xenobiotic** refers to any foreign substance that enters the body. Most drugs are xenobiotics that are used to modulate bodily functions for therapeutic ends. The processes by which biochemical reactions alter drugs within the body are collectively called **drug metabolism** or **drug biotransformation.** Drug biotransformation can alter drugs in four important ways:

- An *active drug* may be converted to an *inactive drug.*
- An *active drug* may be converted to an *active* or *toxic metabolite.*
- An *inactive prodrug* may be converted to an *active drug.*
- An *unexcretable drug* may be converted to an *excretable metabolite* (e.g., to enhance renal or biliary clearance).

The previous chapter introduced the importance of renal clearance in the pharmacokinetics of drugs. Although the biochemical reactions that alter drugs to excretable forms are an essential part of drug metabolism, drug metabolism encompasses more than this one function. This chapter presents the major processes of drug metabolism. Following the case is an overview of the sites of metabolism, focusing principally on the liver. The two major types of biotransformation, the **phase I** and **phase II reactions,** are then described, together with examples of the ways in which these reactions may alter drugs. The chapter concludes with a discussion of the factors that can lead to differences in drug metabolism among individuals.

## Case

Ms B is a 32-year-old Caucasian woman who complains of sore throat and difficulty swallowing for the past 5 days. Physical examination reveals creamy white lesions on the tongue that are identified as oral thrush, a fungal infection. Her history includes sexual activity with multiple partners, inconsistent use of condoms, and continuous use of oral contraceptives for the past 14 years. The presentation suggests a diagnosis of HIV-1 infection, which is confirmed by PCR analysis. Ms B has a low CD4 T-cell count, and is immediately started on a standard anti-HIV regimen that includes the protease inhibitor saquinavir. Her oral thrush resolves with a topical antifungal agent. Despite aggressive therapy, her CD4 cell count continues to decrease, and she presents to her physician several months later with fatigue and a persistent cough. Further investigation leads to a diagnosis of tuberculosis.

### QUESTIONS

1. What concerns would a physician have in devising a drug regimen to treat both Ms B's acute tuberculosis and her underlying HIV disease?
2. One of the first-line drugs in the treatment of tuberculosis is rifampin, which decreases the effec-

tiveness of HIV protease inhibitors. What is the mechanism for this drug–drug interaction?

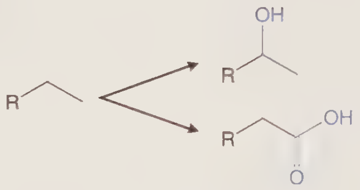
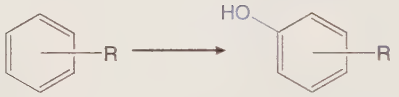
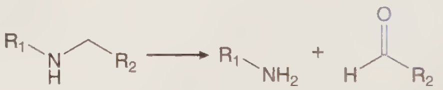
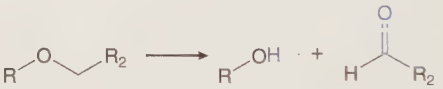
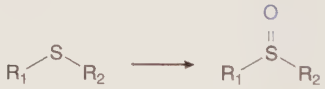
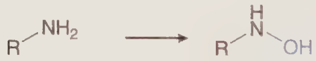
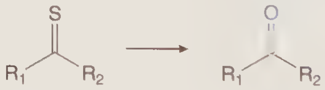
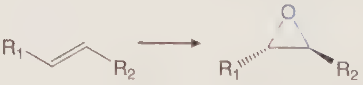
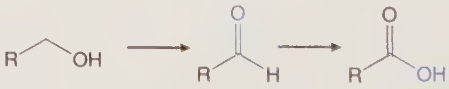
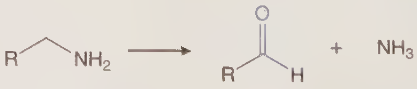
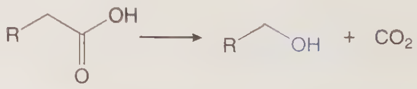
3. Isoniazid is another drug commonly used in the treatment of tuberculosis. Why does Ms B's ethnic background give her physician reason for concern when considering the use of this drug?

## SITES OF DRUG METABOLISM

Many drugs undergo biotransformation before excretion from the body. Most of these biotransformation reactions occur in the liver. Liver cells, or hepatocytes, contain high levels of the enzymes that carry out the two main types of reactions involved in drug metabolism—the **phase I** and **phase II reactions.**

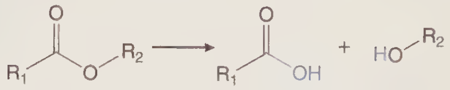
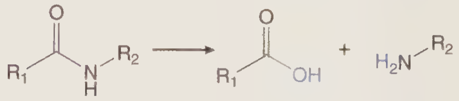
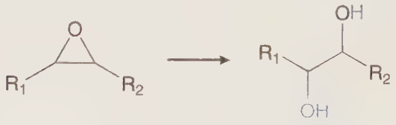
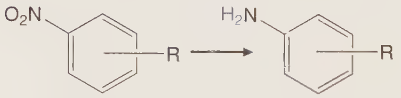
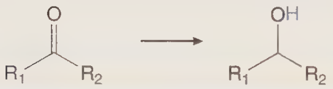
The oral bioavailability of many drugs is increased by making them lipophilic so that they can cross such cell membranes as those of the intestinal mucosa. However, once they are absorbed into the circulation, this same lipophilicity makes renal excretion of the drugs difficult. Clearance by the kidney requires that these drugs be made more hydrophilic so that they can dissolve in the aqueous urine. The phase I and phase II reactions satisfy this requirement. Phase I reactions typically transform the drug into more hydrophilic metabolites by adding or exposing polar functional groups such as hydroxyl (-OH),

**TABLE 4-1** Phase I Reactions

| REACTION CLASS | STRUCTURAL FORMULA | REPRESENTATIVE DRUGS |
|---|---|---|
| **I. Cytochrome P450-Dependent Oxidations** | | |
| 1. Aliphatic Hydroxylation |  | Barbiturates<br>Digitoxin<br>Cyclosporine |
| 2. Aromatic Hydroxylation |  | Propranolol<br>Phenytoin |
| 3. N-Dealkylation |  | Methamphetamine<br>Lidocaine |
| 4. O-Dealkylation |  | Phenacetin<br>Codeine |
| 5. S-Oxidation |  | Phenothiazine<br>Cimetidine |
| 6. N-Oxidation |  | Imipramine<br>Acetaminophen |
| 7. Desulfuration |  | Thiopental |
| 8. Epoxide Formation |  | Aldrin |
| **II. Cytochrome P450-Independent Oxidations** | | |
| 1. Alcohol Dehydrogenation / Aldehyde Dehydrogenation |  | Ethanol<br>Pyridoxine |
| 2. Oxidative Deamination |  | Histamine<br>Norepinephrine |
| 3. Decarboxylation |  | Levodopa |

*(continued)*

**TABLE 4-1** Phase I Reactions *(Continued)*

| REACTION CLASS | STRUCTURAL FORMULA | REPRESENTATIVE DRUGS |
|---|---|---|
| **III. Hydrolysis** | | |
| 1. Ester Hydrolysis |  | Procaine<br>Aspirin<br>Succinylcholine |
| 2. Amide Hydrolysis |  | Procainamide<br>Lidocaine<br>Indomethacin |
| 3. Epoxide Hydrolysis |  | Carbamazepine |
| **IV. Reductions** | | |
| 1. Nitro Reduction |  | Chloramphenicol |
| 2. Dehalogenation | $R{-}X \longrightarrow R{-}H$ | Halothane |
| 3. Carbonyl Reduction |  | Methadone<br>Naloxone |

thiol (-SH), or amine (-$NH_2$) groups (Table 4-1). Such metabolites are often pharmacologically inactive and, without further modification, may be sufficiently polar for excretion. Some products of phase I reactions, however, require further modifications to increase their water solubility. Phase II reactions modify compounds through attachment of hydrophilic groups, such as glucuronate, to create highly polar conjugates (Table 4-2). Many drugs are modified by sequential hydroxylation (phase I) and conjugation (phase II) reactions, but exceptions do exist in which a phase II reaction precedes phase I modification.

The liver is the main organ of drug metabolism. This fact figures prominently in the phenomenon known as the **first-pass effect.** Orally administered drugs are often absorbed unchanged in the gastrointestinal tract and carried directly to the liver via the portal circulation (Fig. 4-1). In this manner, the liver has the opportunity to metabolize drugs before they reach the systemic circulation and, therefore, before they reach their target organs. The first-pass effect must be taken into account when designing dosing regimens because, if hepatic metabolism is extensive, the amount of drug that reaches the target tissue is much less than the amount (dose) that is administered orally (see Chapter 3, Pharmacokinetics). Certain drugs are inactivated so efficiently upon their first pass through the liver that they cannot be administered orally and must be given parenterally. One such drug is the antiarrhythmic lidocaine, which has a bioavailability of only 3% when taken orally.

Although the liver is quantitatively the most important organ in metabolizing drugs, every tissue in the body is capable of drug metabolism to some degree. Particularly active sites include the skin, the lungs, the gastrointestinal tract, and the kidneys. The gastrointestinal tract deserves special mention because this organ, like the liver, can contribute to the first-pass effect by metabolizing orally administered drugs before they reach the systemic circulation.

## PATHWAYS OF DRUG METABOLISM

Hepatic drug metabolism is classically divided into phase I and phase II reactions. Knowledge of the specific mechanisms by which a drug is metabolized can provide insight into the identity of toxic metabolites of the drug and into possible drug–drug interactions.

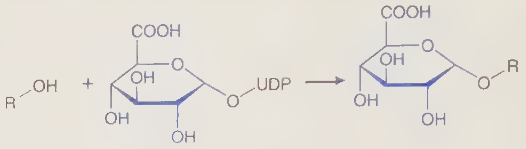
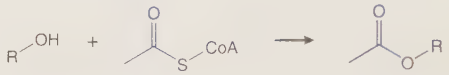
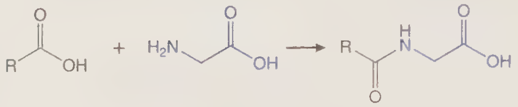
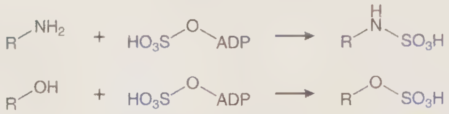
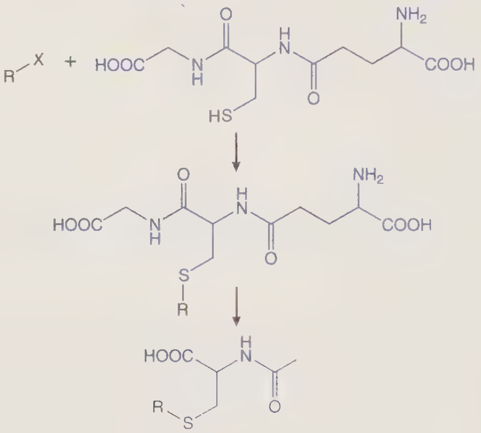
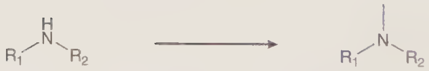
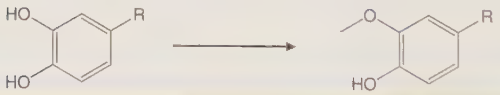
### PHASE I REACTIONS

Phase I reactions involve membrane-associated enzymes found within the endoplasmic reticulum (ER) of hepatocytes and, to a lesser extent, of cells in other tissues. The enzymes that catalyze phase I reactions are typically oxidases; the majority of these enzymes are **hemeprotein monooxygenases** of the **cytochrome P450** class. P450 enzymes are also known as microsomal mixed function oxidases. (The term P450 refers to the 450-nm absorption peak characteristic of these heme proteins when they are treated with carbon monoxide.)

The net result of a cytochrome P450-dependent phase I reaction is:

$$\text{Drug} + O_2 + \text{NADPH} + H^+ \longrightarrow \text{Drug-OH} + H_2O + \text{NADP}^+ \qquad \text{Equation 4-1}$$

## TABLE 4-2 Phase II Reactions

| REACTION CLASS | STRUCTURAL FORMULA | REPRESENTATIVE DRUGS |
|---|---|---|
| 1. Glucuronidation |  | Diazepam<br>Digitoxin<br>Digoxin |
| 2. Acetylation |  | Isoniazid<br>Sulfonamides |
| 3. Glycine Conjugation |  | Salicylic Acid<br>Benzoic Acid |
| 4. Sulfate Conjugation |  | Estrone<br>Methyldopa |
| 5. Glutathione Conjugation/ Mercapturic Acid Biosynthesis |  | Ethacrynic Acid<br>Bromobenzene |
| 6. N-Methylation |  | Methadone<br>Norepinephrine |
| 7. O-Methylation |  | Catecholamines |

The reaction proceeds when the drug binds to the oxidized ($Fe^{3+}$) cytochrome P450 to form a complex, which is then reduced in two sequential oxidation/reduction steps as outlined in Figure 4-2A. Nicotinamide adenine dinucleotide phosphate (NADPH) donates the electrons in both of these steps via a flavoprotein reductase. In the first step, the donated electron reduces the cytochrome P450-drug complex. In the second step, the electron reduces molecular oxygen to form an activated oxygen-cytochrome P450-drug complex. Finally, as the complex dissociates, the activated oxygen is shifted to the drug, resulting in the formation of the oxidized drug product and recycling oxidized cytochrome P450 in the process. The mechanism of these reactions is illustrated in Figure 4-2B.

The liver cytochrome P450 oxidases exhibit broad substrate specificity (Table 4-1). This is in part caused by the activated oxygen of the complex, which is a powerful oxidizing agent that can react with a variety of substrates. In addition, the cytochrome P450 enzymes belong to a superfamily whose members, or subtypes, are known as P450 isozymes. The family names of the cytochrome P450 enzymes are designated by "CYP" followed by the number of the family, capital letter of the subfamily, and an additional number to identify the specific enzyme (e.g., CYP3A4). Many of these isozymes have partially overlapping specificities that together allow the liver to recognize and metabolize a wide array of xenobiotics.

Although P450-mediated reactions account for the majority of phase I biotransformations, a number of significant

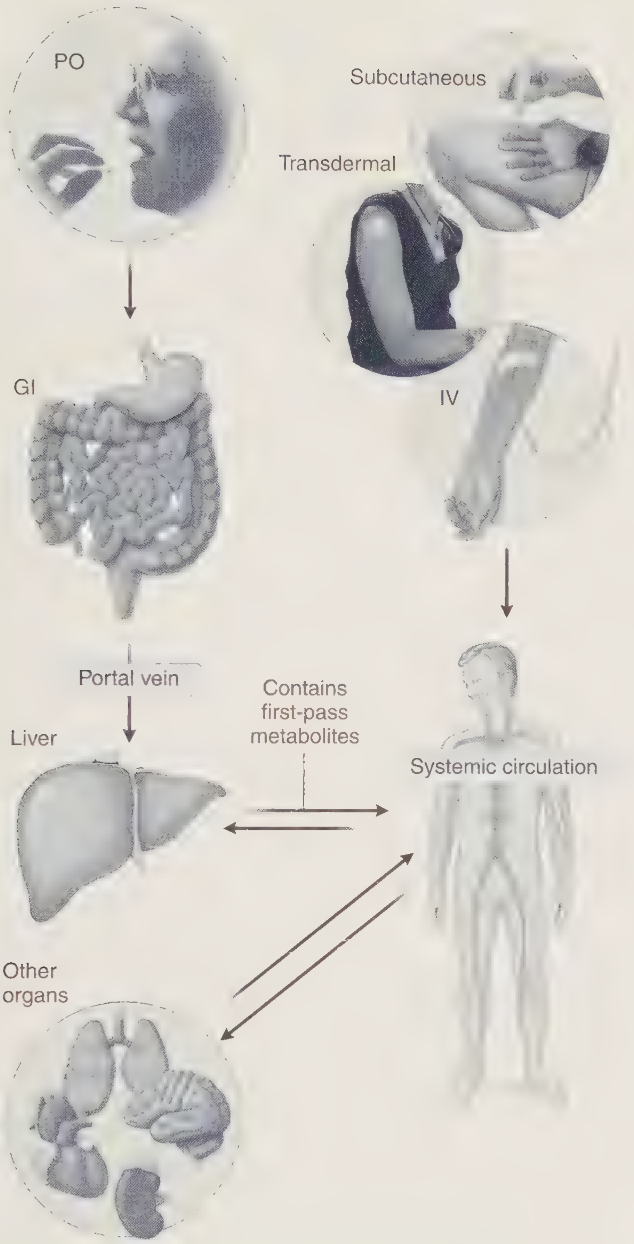


**Figure 4-1. Portal Circulation and the First-Pass Effect.** Drugs administered by mouth (*per os*, or PO) are absorbed in the gastrointestinal tract (GI) and then delivered, via the portal vein, to the liver. This pathway allows the liver to metabolize drugs before they reach the systemic circulation, a process responsible for the **first-pass effect.** In contrast, drugs that are administered intravenously (IV), transdermally, or subcutaneously enter the systemic circulation directly and can reach their target organs before hepatic modification. The first-pass effect has important implications for bioavailability; the oral formulation of a drug that undergoes extensive first-pass metabolism must be administered in a much larger dose than the equivalent IV formulation of the same drug.

phase I reactions occur through other pathways. Some of these pathways, like the P450-dependent reactions, serve to oxidize lipophilic molecules. Because the P450-independent oxidations do not require P450 enzymes, these reactions do not occur exclusively in the ER membrane. This mechanism allows local hydrolysis of ester-containing and amide-containing drugs (e.g., local anesthetics) at their site of administration, thus limiting systemic toxicity. Another important P450-independent phase I enzyme is monoamine oxidase (MAO); this enzyme is responsible for the oxidation of amine-containing endogenous and exogenous compounds such as catecholamines and tyramine (see Chapter 8, Adrenergic Pharmacology).

## PHASE II REACTIONS

Phase II reactions provide a second set of mechanisms for modifying insufficiently polar compounds to more hydrophilic forms for excretion (Fig. 4-3). Substrates for these reactions include both metabolites of phase I reactions and compounds that already contain chemical groups appropriate for conjugation such as hydroxyl (-OH), amine (-$NH_2$), or carboxyl (-COOH) moieties. These substrates are coupled by transfer enzymes to endogenous substances (e.g., glucuronic acid and its derivatives, sulfuric acid, acetic acid, or amino acids) in reactions that involve high-energy intermediates (Table 4-2). Phase II transferases are located in both the cytosol and the ER of hepatocytes. Virtually all phase II metabolites are pharmacologically inactive.

Phase II reactions are important clinically in the case of neonates, who have not yet fully developed the capacity to carry out this set of reactions. The phase II enzyme UDP-glucuronyl transferase (UDP-GT) is responsible for conjugating bilirubin in the liver and facilitating its excretion. The developmental deficiency of this enzyme at birth puts infants at risk for neonatal jaundice, which results from increased serum levels of unconjugated bilirubin. Neonatal jaundice is a problem because neonates have not only underdeveloped phase II capabilities but also an undeveloped blood–brain barrier. Unconjugated bilirubin is water-insoluble and lipophilic, so it binds easily to the unprotected neonate brain and is capable of causing significant damage to the central nervous system. This pathologic condition is known as kernicterus (bilirubin encephalopathy). Prophylactic treatments for kernicterus include: (1) phototherapy with 450-nm light, which converts bilirubin to an isomer that is more rapidly excreted, and (2) administration of the barbiturate phenobarbital, which acts to induce UDP-GT and thereby to reduce serum levels of unconjugated bilirubin.

## INDUCTION AND INHIBITION

The use of phenobarbital to prevent neonatal jaundice demonstrates that drug metabolism can be influenced by the levels of phase I and phase II enzymes that are present. Although some of the P450 isozymes are constitutively active, others can be **induced** or **inhibited** by different compounds. Induction or inhibition can be incidental (a side effect of a drug) or deliberate (the desired effect of therapy). The mechanism of enzyme induction can involve either increasing the rate of enzyme synthesis (through increased transcription and translation) or decreasing the rate of enzyme degradation; either effect results in accelerated drug metabolism. Compounds capable of inducing liver enzymes include many drugs as well as some environmental pollutants and industrial chemicals. Some of the commonly prescribed drugs that affect P450-dependent drug metabolism are listed in Table 4-3.

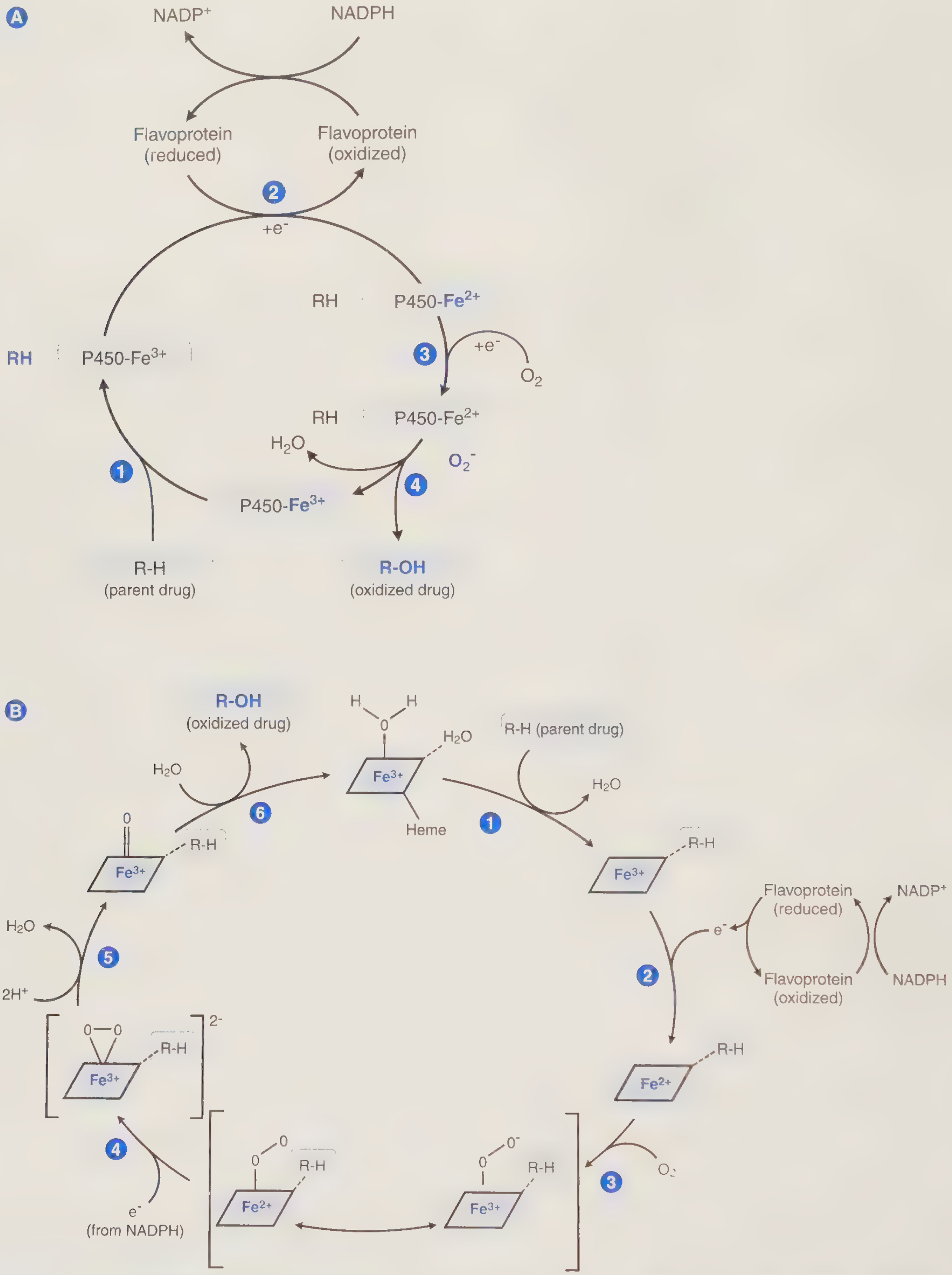

A
NADP+
NADPH
Flavoprotein (reduced)
Flavoprotein (oxidized)
2
+e-
RH
P450-Fe2+
RH
P450-Fe3+
3
+e-
O2
RH
P450-Fe2+
H2O
O2-
1
4
P450-Fe3+
R-H (parent drug)
R-OH (oxidized drug)
B
R-OH (oxidized drug)
H H O
H2O
R-H (parent drug)
H2O
Fe3+
Heme
H2O
6
1
O
R-H
Fe3+
R-H
Fe3+
Flavoprotein (reduced)
NADP+
H2O
5
e-
2
2H+
Flavoprotein (oxidized)
NADPH
O—O
2-
R-H
Fe3+
R-H
Fe2+
4
O
O
R-H
O
O-
R-H
O2
3
Fe2+
Fe3+
e-
(from NADPH)

Figure 4-2. **Cytochrome P450-Mediated Drug Oxidation.** Phase I drug metabolism reactions involve a system of hepatic cytochrome P450 microsomal enzymes that catalyze the oxidation of drugs to more soluble metabolites. **A.** An overview of the phase I reaction involves a set of oxidation/reduction steps in which an iron moiety in the P450 enzyme acts as an electron carrier to transfer electrons from NADPH to molecular oxygen. The reduced oxygen is then transferred to the drug, resulting in an additional -OH group on the now-oxidized drug (for this reason, cytochrome P450 enzymes are sometimes referred to colloquially as "oxygen guns"). The addition of the -OH group results in increased drug hydrophilicity and, therefore, an increased rate of drug excretion. **B.** The detailed mechanism of the phase I P450 reaction can be divided into six steps: **(1)** drug complexes with oxidized cytochrome P450; **(2)** NADPH donates an electron to flavoprotein reductase, which reduces the P450-drug complex; **(3 and 4)** oxygen joins the complex, and NADPH donates another electron, creating the activated oxygen–cytochrome P450-substrate complex; **(5)** iron is oxidized, with the loss of water; and **(6)** the oxidized drug product is formed. There are multiple isoforms of cytochrome P450; each isoform has a somewhat different specificity for substrates (such as drugs).

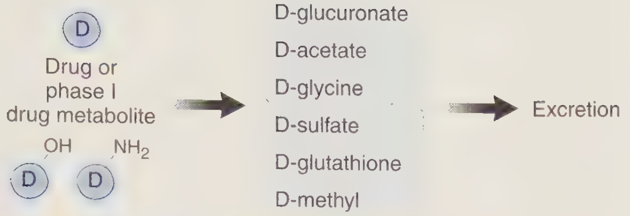


**Figure 4-3. Phase II Conjugation Reactions.** Phase II reactions are the second major pathway for drug metabolism in hepatocytes. In these reactions, a drug (represented by D) or phase I drug metabolite (represented by D-OH and D-$NH_2$) is conjugated to an endogenous moiety. Glucuronate, a sugar, is the most common group that is conjugated to drugs, but conjugations of acetate, glycine, sulfate, glutathione, and methyl groups are also common. The addition of one of these moieties makes the resulting drug metabolite more hydrophilic, and hence enhances drug excretion.

There are multiple consequences of P450 enzyme induction. For example, carbamazepine, an antiepileptic drug, not only induces the P450 isozyme CYP3A4 but also is metabolized by CYP3A4. Hence, carbamazepine hastens its own metabolism by inducing CYP3A4. In addition, CYP3A4 is responsible for metabolizing more than 60% of all clinically prescribed drugs. Should such a drug be coadministered with carbamazepine, its metabolism would also be increased. This situation can be problematic, because the increased CYP3A4 activity can reduce drug concentrations below their therapeutic levels if the standard dose of drug is administered. This point is relevant to Ms B's case: the administration of rifampin in conjunction with her HIV therapy could be detrimental, because rifampin induces the CYP3A4 isozyme that also metabolizes such protease inhibitors as saquinavir. Finally, induction of phase I P450 enzymes can also result in the production of toxic levels of reactive drug metabolites, resulting in tissue damage or other side effects.

Just as certain compounds can induce cytochrome P450 isozymes, other compounds can inhibit these enzymes. An important clinical consequence of enzyme inhibition is the decreased metabolism of drugs that are metabolized by the inhibited enzyme. Such inhibition can both allow drug levels to reach toxic concentrations and also prolong the presence of active drug in the body.

**TABLE 4-3** Some Important Pharmacologic Inducers and Inhibitors of Cytochrome P450 Enzymes

| INDUCERS | INHIBITORS |
|---|---|
| Phenytoin | Omeprazole |
| Carbamazepine | Disulfiram |
| Barbiturates | Erythromycin |
| Rifampin | Valproic Acid |
| Ritonavir | Isoniazid |
| Chronic ethanol toxicity | Cimetidine |
| Griseofulvin | Ciprofloxacin |
| | Acute ethanol toxicity |

Enzyme inhibition can be achieved in a variety of ways. For example, cimetidine, a widely used histamine-2 receptor antagonist (see Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease), has a nitrogen moiety that binds to the heme iron in the active site of P450 isozymes; this binding prevents the metabolism of coadministered drugs by competitive inhibition. Box 4-1 illustrates the clinical relevance of a common drug interaction involving cimetidine. Secobarbital, a barbiturate, irreversibly inhibits the P450 complex by alkylation of the enzyme. On occasion, the inhibition of P450 enzymes can be used to therapeutic advantage: for example, the protease inhibitor lopinavir alone cannot achieve therapeutic levels because of extensive first-pass metabolism, but coadministration of lopinavir with the P450 CYP3A inhibitor ritonavir (also a protease inhibitor) allows lopinavir to reach efficacious concentrations.

## ACTIVE AND TOXIC METABOLITES

Knowing how a given agent is metabolized may bear significantly on the choice of what agent to prescribe. This is true both when the metabolite is active, in which case the administered agent may be acting as a **prodrug,** and when the agent has **toxic metabolites.**

*Prodrugs are inactive compounds that are metabolized by the body into their active, therapeutic forms.* For example, an effective treatment for the symptoms of Parkinson's disease is replacement of the neurotransmitter dopamine, which is deficient in this progressive neurodegenerative disorder. To exert its effect, dopamine must reach the brain. This presents a problem, because the neurotransmitter itself does not cross the blood–brain barrier. To overcome this obstacle, levodopa—the metabolic precursor to dopamine—is administered instead of dopamine, because levodopa can cross the blood–brain barrier. Once in the brain, the prodrug levodopa is metabolized to the active "drug" dopamine by the endogenous enzyme DOPA decarboxylase. In this way, administration of a prodrug results in targeted treatment of the disease (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission, for a more extensive discussion of treatments for Parkinson's disease).

The metabolism of many xenobiotics can produce reactive intermediates that may injure body organs. The detrimental effects of certain drug biotransformations often become visible only after endogenous systems of detoxification are overwhelmed, putting into play alternative pathways that generate cytotoxic or carcinogenic metabolites. One clinically significant example is that of acetaminophen, a commonly used analgesic and antipyretic. In its therapeutic concentration range, acetaminophen is processed predominantly by phase II-mediated glucuronidation and sulfation, with the conjugate products accounting for approximately 95% of the total excreted metabolites. P450 enzymes N-hydroxylate a small fraction to a reactive intermediate that is immediately conjugated to glutathione. However, when the level of acetaminophen exceeds the therapeutic range, not only can the glucuronidation and sulfation pathways be saturated, but also the stores of glutathione in the liver can be depleted. This can cause build-up of the reactive intermediate—*N*-acetyl-benzoquinoneimine—that is normally taken up and transformed by glutathione. The reactive intermediate is electrophilic and tends to react with nucleophilic groups on pro-

### BOX 4-1. Application to Therapeutic Decision-Making: Drug Interactions with Warfarin

(by Vivian Gonzalez Lefebre and Robert H. Rubin)

Warfarin is a frequently prescribed anticoagulant whose metabolism can be altered by many other drugs, sometimes with fatal consequences. Consider the following clinical scenario.

Mrs. Johnson, a 75-year-old woman, is sitting in her chair at home talking with her son when she suddenly develops a severe headache and loses consciousness. Her son immediately calls 911, and an ambulance transports her to the ER. Soon after arriving in the ER, Mrs. Johnson regains consciousness, but she cannot comprehend any words and is unable to move her right arm and leg. A computed tomographic scan reveals an intraparenchymal hemorrhage in the left frontal lobe extending to the ventricles and subarachnoid space. Shortly thereafter, it is discovered that Mrs. Johnson has a 5-year history of atrial fibrillation that has been treated with warfarin and a β-blocker and that she has recently (5 days ago) started taking cimetidine for symptomatic heartburn. Her INR (International Normalized Ratio, a measure of clotting cascade function) is measured at 11.3 (cf., a therapeutic INR is 2.0–3.0).

How could drug interactions have led to this patient's stroke? In general, drugs can interact with warfarin by pharmacodynamic or pharmacokinetic mechanisms. Pharmacodynamic mechanisms for drug interactions with warfarin include synergism (e.g., impaired hemostasis, reduced clotting factor synthesis), competitive antagonism (e.g., use of vitamin K), and altered substrate availability (e.g., decreased vitamin K absorption, an effect that accompanies the use of some antibiotics). Pharmacokinetic mechanisms for drug interactions with warfarin include primarily hepatic enzyme induction, hepatic enzyme inhibition, and reduced plasma protein binding. Some drugs may interact with warfarin by more than one mechanism. Warfarin has one of the longest lists of potential drug interactions, and thus, its effects must be closely monitored by frequent measurements of the INR. Refer to Chapter 21, Pharmacology of Hemostasis and Thrombosis, for a detailed discussion of the mechanism of action of warfarin.

Cimetidine, a frequently used histamine-2 receptor antagonist (see Chapter 44), is a potent inhibitor of several P450 enzymes. Inhibition of P450 activity by cimetidine leads to increased plasma levels of a number of drugs that are metabolized by the same P450 enzymes, including warfarin. In Mrs. Johnson's case, it is likely that concomitant administration of warfarin and cimetidine has predisposed her to an intracranial hemorrhage by causing a sharp increase in the plasma level of warfarin and a consequent increase in her bleeding risk. This is an example of a pharmacokinetic interaction involving warfarin. Clearly, her INR should have been monitored more closely after the addition of cimetidine to her therapeutic regimen.

teins to produce covalent protein derivatives. Such intermediate-protein complexes are highly toxic to the liver and can lead, in the case of acetaminophen overdose, to fulminant hepatotoxicity and death. One antidote is *N*-acetylcysteine, a glutathione precursor that replenishes stores of the antioxidant. If *N*-acetylcysteine is administered within 8 to 16 hours following the overdose, it may be lifesaving.

## INDIVIDUAL FACTORS AFFECTING DRUG METABOLISM

For several reasons, the rates of biotransformation reactions may vary greatly from one person to another. The most important of these factors are discussed below.

### PHARMACOGENOMICS

The effects of genetic variability on drug metabolism are an important part of the new science of pharmacogenomics (see Chapter 52, Pharmacogenomics). Certain populations exhibit polymorphisms or mutations in one or more enzymes of drug metabolism, changing the rate of some of these reactions or eliminating others altogether. These pharmacogenetic differences must be taken into account in therapeutic decision-making and drug dosing.

For example, one in every 2,000 Caucasians carries a genetic alteration in the plasma enzyme cholinesterase, which (among other functions) metabolizes the muscle relaxant succinylcholine. This altered form of the enzyme has an approximately 1,000-fold reduced affinity for succinylcholine, resulting in slowed elimination and prolonged circulation of the active drug. Should a sufficiently high plasma concentration of succinylcholine be reached, respiratory paralysis and death can occur unless the patient is supported with artificial respiration until the drug is cleared.

A similar situation can occur with the drug isoniazid, one of the drugs considered for treatment of Ms B's tuberculosis. Genetic variability, in the form of a widespread autosomal recessive trait that results in decreased synthesis of an enzyme, causes the metabolism of this drug to be slowed in certain subsets of the U.S. population. The enzyme in question is *N*-acetyl transferase, which inactivates isoniazid in a phase II acetylation reaction. The "slow acetylator" phenotype is expressed by 45% of whites and blacks in the U.S. and by some Europeans living in high northern latitudes. The "fast acetylator" phenotype is found in >90% of Asians and in Inuits in the U.S. Blood levels of isoniazid are elevated fourfold to sixfold in slow acetylators relative to fast acetylators. Moreover, because the free drug acts as an inhibitor of P450 enzymes, slow acetylators are more susceptible to adverse drug interactions. If Ms B should express the "slow acetylator" phenotype, and her dose of isoniazid is not adjusted accordingly, then the addition of isoniazid to her drug regimen could potentially have a toxic effect.

## AGE AND GENDER

Drug metabolism can also differ among individuals as a result of age and gender differences. Many reactions of biotransformation are slowed in both young children and the elderly. At birth, neonates are capable of carrying out most phase I reactions; however, both phase I and phase II enzyme systems mature gradually over the first 2 weeks of life and throughout childhood. Recall that neonatal jaundice results from a deficiency of the phase II enzyme UDP-GT. Another example of a phase II enzyme deficiency that puts infants at risk for toxicity is the so-called **gray baby syndrome,** an iatrogenic condition that was seen more often in the past than at present. *Hemophilus influenza* infections in infants were once treated with chloramphenicol, an antibiotic that requires a phase I modification followed by a phase II conjugation to be excreted. The phase I metabolite of chloramphenicol is toxic; if this metabolite fails to undergo phase II modification, it can build up in the plasma and may reach toxic concentrations. Toxic levels of the metabolite can cause neonates to suffer from shock and circulatory collapse, leading to the pallor and cyanosis that give the syndrome its name.

In the elderly, a general decrease in metabolic capacity is observed. This decline in function has been attributed to age-related decreases in liver mass, hepatic enzyme activity, and hepatic blood flow. Aging seems to affect P450 enzymes preferentially; alternate metabolic pathways, including those involving phase II enzymes, seem to be spared. As a result, particular care must be taken in prescribing drugs for the elderly.

Although the mechanisms are not well understood, there is some evidence for gender differences in drug metabolism. Decreased oxidation of ethanol, estrogens, benzodiazepines, and salicylates has been reported anecdotally in women relative to men. These gender-dependent differences may be related to androgenic hormone levels.

## DIET AND ENVIRONMENT

Both diet and environment may alter drug metabolism by inducing or inhibiting enzymes of the P450 system. An interesting example is grapefruit juice, which contains psoralen derivatives and flavonoids that inhibit the CYP3A4 isozyme in the small intestine and thereby decrease first-pass metabolism. This "grapefruit juice effect" is important when grapefruit juice is ingested together with drugs that are metabolized by this enzyme. Such drugs include some protease inhibitors, macrolide antibiotics, HMG CoA reductase inhibitors, and calcium-channel blockers. One of the protease inhibitors metabolized by CYP3A4 is saquinavir, which is pertinent to the case of Ms B. Because Ms B's drug regimen includes saquinavir, this interaction should be brought to her attention to prevent possible toxic plasma levels of saquinavir caused by the simultaneous ingestion of grapefruit juice.

Because many endogenous substances used in phase II conjugation reactions are ultimately derived from the diet, nutrition can affect drug metabolism by altering the pool of such substances available to the conjugating enzymes. Pollutant exposures may produce similarly dramatic effects on drug metabolism; two of the most common effects include P450 enzyme induction by cigarette smoke and by polycyclic aromatic hydrocarbons (from petroleum products).

## METABOLIC DRUG INTERACTIONS

Drugs may potentially affect oral bioavailability, plasma protein binding, hepatic metabolism, and renal excretion of coadministered drugs. Of these drug–drug interactions, the effects on biotransformation have special clinical importance. The concept of P450 enzyme induction and inhibition has already been introduced. A common clinical situation that must take this type of drug–drug interaction into consideration is the prescription of certain antibiotics to women on oral contraceptives. For example, enzyme induction by the antibiotic rifampin causes oral contraceptives to be ineffective at standard doses, and other means of birth control are recommended for that time period. Ms B should be made aware of this interaction if rifampin is added to her therapeutic regimen. (The herbal supplement, St. John's wort, has a similar effect on oral contraceptive levels.) Another mechanism associated with enzyme induction is tolerance, which can occur when a drug induces its own metabolism and, thus, reduces its efficacy over time (see the discussion of carbamazepine above and the discussion of tolerance in Chapter 16, Pharmacology of Chemical Dependence and Addiction).

Competitive substrate inhibition and irreversible enzyme inactivation are also common clinical situations that require attention. An example of competitive substrate inhibition is the coadministration of the selective estrogen receptor modulator (SERM) tamoxifen, a drug used in the treatment of breast cancer, and warfarin, an anticoagulant (see Chapter 27, Pharmacology of Reproduction, and Chapter 21, Pharmacology of Hemostasis and Thrombosis, respectively). These two drugs compete for the same metabolizing enzyme, CYP3A4. When the drugs are coadministered, the dose of warfarin must be reduced to prevent the possibility of life-threatening bleeding. The fact that warfarin has a narrow therapeutic index necessitates careful monitoring of the drug's plasma levels, when administered either alone or with other drugs. Box 4-1 illustrates this principle for the drug interaction involving warfarin and cimetidine. An example of irreversible enzyme inactivation is aspirin, which irreversibly acetylates cyclooxygenase. This results in inhibition of platelet eicosanoid signaling (see Chapter 40, Pharmacology of Eicosanoids) and, therefore, decreased platelet function. Patients undergoing surgery must discontinue aspirin several days before surgery to avoid unwanted bleeding.

## DISEASES AFFECTING DRUG METABOLISM

Many disease states can affect the rate and extent of drug metabolism in the body. Because the liver is the main site of biotransformation, many liver diseases significantly compromise drug metabolism. Hepatitis, cirrhosis, cancer, hemochromatosis, and fatty infiltration of the liver all impair cytochrome P450s and other hepatic enzymes that are crucial to drug metabolism. As a result of this slowed metabolism, the levels of the active forms of many drugs may be significantly higher than intended and thereby cause toxic effects. Thus, the doses of many drugs may need to be lowered for individuals with hepatic disease.

Concomitant cardiac disease can also affect drug metabolism. The rate of metabolism of many drugs, such as the antiarrhythmic lidocaine and the opioid morphine, is dependent on drug delivery to the liver via the bloodstream. Because blood flow is commonly compromised in cardiac disease, there must be heightened awareness of the potential for supratherapeutic levels of drugs in patients with heart failure. In addition, some antihypertensive agents selectively reduce blood flow to the liver and, therefore, can increase the half-life of such a drug as lidocaine, leading to potentially toxic levels.

Thyroid hormone regulates the basal metabolic rate of the body, which, in turn, affects drug metabolism. Hyperthyroidism can increase the rate of metabolism of some drugs, whereas, hypothyroidism can do the opposite. Other conditions, such as pulmonary disease, endocrine dysfunction, and diabetes, are also thought to affect drug metabolism, but the mechanisms for these effects are not yet completely understood.

## Conclusion and Future Directions

This chapter has reviewed a number of issues relating to drug metabolism, including the sites at which biotransformation takes place, the types of reactions that occur, and individual factors that can affect those reactions. The case of Ms B illustrates the clinical implications of drug metabolism, including the possible influences of ethnicity and drug–drug interactions on pharmacologic therapy. Understanding drug metabolism allows the principles of biotransformation to be applied in the design and use of therapeutics. As pharmacogenomics and rational drug design take pharmacology research into the future, biotransformation will become increasingly important in clinical practice and render the pharmacologic treatment of disease more individualized, efficacious, and safe. This topic is discussed in Chapter 52, Pharmacogenomics.

## Suggested Readings

Dresser GK, Spence JD, Bailey DG. Pharmacokinetic–pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet 2000;38:41–57. (*Discusses CYP3A4 inhibition by various substances, including grapefruit juice.*)

www.pharmacology.about.com. (*This website contains a comprehensive list of drugs whose levels are potentially affected by ingestion of grapefruit juice.*)

Flexner C. Drug therapy: HIV protease inhibitors. N Engl J Med 1998;338:1281–1292. (*Reviews the use of protease inhibitors in HIV therapy, including relevant issues of drug interactions and viral resistance.*)

Singh BN. Effects of food on clinical pharmacokinetics. Clin Pharmacokinet 1999;37:213–255. (*Reviews the known interactions between food and commonly prescribed agents.*)

# II

# Principles of Neuropharmacology

# IIA

# Fundamental Principles of Neuropharmacology

# 5

# Principles of Cellular Excitability and Electrochemical Transmission

Michael Tsan Ty and Gary R. Strichartz

Introduction
Cellular Excitability
    Ohm's Law
    Ion Channels
    Channel Selectivity, the Nernst Equation and the Resting Potential
    The Goldman Equation
    The Action Potential
Pharmacology of Ion Channels
Electrochemical Transmission
    Synaptic Vesicle Regulation
    Postsynaptic Receptors
    Transmitter Metabolism and Reuptake
Conclusion
Suggested Reading

## INTRODUCTION

Cellular communication is essential for the effective functioning of any complex multicellular organism. The major mode of intercellular communication is the transmission of chemical substances, such as neurotransmitters and hormones. In excitable tissues, such as nerves and muscles, rapid intracellular communication relies on the propagation of electrical signals—action potentials—along the plasma membrane of the cell. Both chemical and electrical transmission commonly involve the movement of ions across the plasma membrane that separates the cell from its environment or across the membranes of such internal organelles as the endoplasmic reticulum or mitochondria. Ionic movements can directly change the cytoplasmic concentration of ions, such as $Ca^{2+}$, that are key regulators of such biochemical and physiologic processes as phosphorylation and contraction. Ionic movements also change the electrical potential across the membrane through which the ions flow, thus regulating various **voltage-dependent** functions, such as the opening of other ion channels. Some of these events are brief, with durations and actions of several milliseconds (0.001 sec). Others may take many seconds, with biochemical consequences that may persist for minutes or hours. Even gene expression can be regulated by changes in ion concentrations, resulting in long-term changes in cellular physiology, growth, differentiation, and other processes.

Many drugs modify chemical or electrical signaling to increase or decrease cellular excitability and electrochemical transmission. To appreciate how such drugs work, the present chapter explains the electrochemical foundations that underlie these phenomena. These general principles are applicable to many areas of pharmacology, including Chapters 7–9, Principles of Autonomic and Peripheral Nervous System Pharmacology; Chapters 10–16, Principles of Central Nervous System Pharmacology; and Chapter 17, Pharmacology of Cardiac Rhythm.

## CELLULAR EXCITABILITY

**Excitability** refers to the ability of a cell to generate and propagate electrical **action potentials.** Neuronal, cardiac, smooth muscle, skeletal muscle, and many endocrine cells have an excitable character. Action potentials may propagate over large distances, as in peripheral nerve axons that conduct over several meters, or they may stimulate activity in cells of much smaller size, such as interneurons 30–50 μm in length that are contained within one autonomic ganglion. The function of action potentials differs depending on the cells where they occur. In propagating along axons, waves of action potentials carry encoded information with fidelity over long distances. Within a small cell, action potentials excite the whole cell at once, with a concomitant increase in intracellular ions such as $Ca^{2+}$ and the subsequent rapid release of chemical transmitter molecules or of hormones. These chemicals then travel to specific receptors, near or far from the releasing cell, to effect **chemical transmission,** which is discussed in the second part of this chapter.

*Cellular excitability is fundamentally an electrical event.* Therefore, an understanding of basic electricity is necessary to explain the biological processes of excitability and synaptic transmission. The following sections present basic principles of electricity as applied to two important cellular constituents: the plasma membrane and ion-selective channels.

## OHM'S LAW

The magnitude of a current (I, measured in amps) flowing between two points is determined by the potential difference (V, measured in volts) between those two points and the resistance to current flow (R, measured in Ohms):

$I = V/R$ Equation 5-1a

For example, current may flow from the extracellular to the intracellular compartment in response to a potential difference (also known as a voltage difference) across the plasma membrane. Voltage can be thought of as a potential energy or the propensity for charge to flow from one area to another. Resistance is the obstacle to this flow. Decreased resistance allows greater ion flow and, therefore, increased current (current has units of charge/time). When this relationship, known as Ohm's law, is applied to biological membranes such as the plasma membrane, it is often easier to replace the electrical resistance by its reciprocal, the conductance [g, measured in reciprocal Ohms, or Siemens (S)]:

$I = gV$ Equation 5-1b

For simplicity, assume that all resistive elements in the cell membrane behave in an "ohmic way;" that is, that their current-voltage (I–V) relationship is described by Equation 5-1b. In this case, the I–V relationship is linear, with a slope given by the conductance, g. Figure 5-1 represents the transmembrane current (I) measured at different transmembrane potentials (V) in a hypothetical cell. The slope of the I–V curve represents the conductance. From a conceptual perspective, current increases as voltage increases because a greater voltage results in a greater potential energy difference between two points, which in turn favors an increased rate of charge movement between the points.

The convention used in most texts and in this chapter is that the voltage across a membrane is expressed as the difference between the intracellular and the extracellular potential ($V_m = V_{in} - V_{out}$). For most normal cells, $V$ is negative when the cell is at rest ($V_{in} < V_{out}$). The membrane is termed **hyperpolarized** when $V$ is more negative than at rest, and it is described as **depolarized** when $V$ is more positive than at rest. Positive charge moving from inside to outside is called outward current, and is represented graphically by positive values; whereas, inward current is represented by negative values (movement of negative charge is defined in the exact opposite way). Current is conventionally defined with respect to the direction in which positive charge flows.

## ION CHANNELS

How does current actually flow across a cell membrane? Biological membranes are composed of two phospholipid monolayers arranged in opposition to one another (Fig. 5-2). Pure lipid membranes are virtually impermeable to most polar or charged substances. From an electrical perspective, the lipid

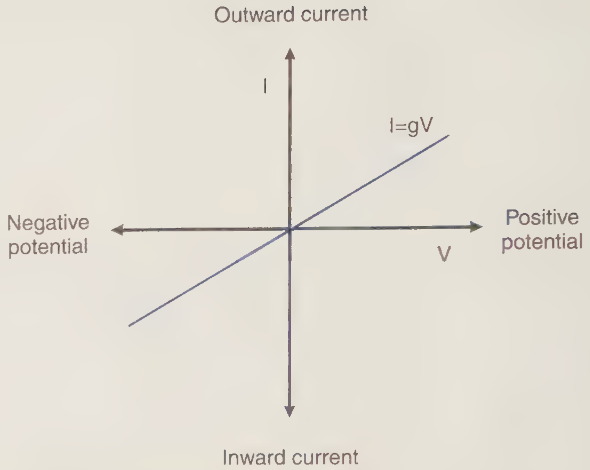


**Figure 5-1. Ohm's Law.** Ohm's law states that there is a linear relationship between current (I) and voltage (V), and that the slope of the I/V plot gives the conductance (g). By convention, outward current is the flow of positive charge from inside the cell to outside the cell. Transmembrane potential is defined by the difference in potential (voltage) between the inside and the outside of the cell; for most cells, the resting voltage inside the cell is negative relative to that outside the cell. Conductance, g, is the reciprocal of resistance.

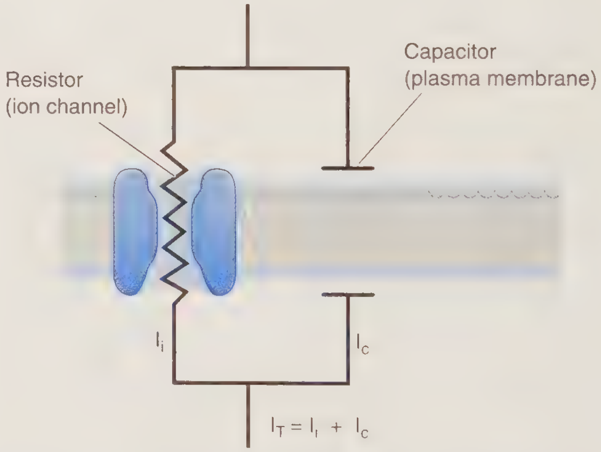


**Figure 5-2. Electrical Circuit Model of the Cell Membrane.** The cell membrane can be modeled as a simple electrical circuit containing a resistor and a capacitor. Ion-selective channels function as resistors (identical to conductors) through which ions can flow down their electrochemical gradient. The lipid bilayer acts as a capacitor by maintaining a separation of charges between the extracellular and intracellular spaces. This circuit (referred to as an RC, or resistor-capacitor, circuit) changes the timing between the flow of charges across the membrane (current) and changes in transmembrane potential (voltage), because the lipid bilayer, acting as a capacitor, stores some of the charge that passes across the membrane. Time is required to store this charge; therefore, the initial change in voltage associated with a step of current is slow. As the capacitor (lipid bilayer) fills with charges and the voltage change grows, more of the charge passes through the resistor, until a new steady state is reached and the current–voltage relationship becomes more linear. ($I_c$, capacitor current; $I_i$, ionic current; $I_T$, total current.)

bilayer acts as a capacitor by maintaining charge separation between the extracellular and intracellular ions. To allow the passage of ions that carry electrical current, ion channels or pores are embedded in the membrane. Most ion channels discriminate among the various types of ions, and most also remain closed until specific signals dictate their opening. From an electrical perspective, a set of ion channels is a variable conductor—it provides many individual conductances for ion flow between the extracellular and intracellular environments. The magnitude of the overall conductance depends on the fraction of channels in the open state and the conductance of a single open channel.

## CHANNEL SELECTIVITY, THE NERNST EQUATION AND THE RESTING POTENTIAL

By itself, the hypothetical I–V relation in Figure 5-1 does not explain the electrical behavior of most real cells. If a cell behaved according to Equation 5-1, then in the absence of an externally applied current, the potential difference across the membrane would be zero. Instead, most cells maintain a negative potential difference across their plasma membrane. This voltage difference is most pronounced in neuronal and cardiac ventricular cells, where a resting potential (the voltage difference across the membrane in the absence of external stimuli) of –60 to –80 mV can be recorded. The resting potential results from three factors: (1) an unequal distribution of positive and negative charges on each side of the plasma membrane; (2) a difference in permeability of the membrane to the various cations and anions; and (3) the action of active (energy-requiring) and passive pumps that help to maintain the ion gradients. The effects of these three interrelated factors can be best explained with an example.

Consider the case when there are only potassium ($K^+$) and protein-bound anions ($A^-$) inside the cell, and no other ions outside the cell (Fig. 5-3). If this cell membrane is permeable to potassium alone, then $K^+$ will flow outward, while A will remain inside. The $K^+$ flows outward because of a **chemical gradient;** that is, $K^+$ efflux is favored because the $K^+$ concentration inside the cell is greater than that outside the cell. Efflux of the anion, $A^-$, would also be favored by its chemical gradient, but the absence of transmembrane channels permeable to $A^-$ prevents this anion from flowing across the membrane. (In other words, the membrane is impermeable to $A^-$.) Because of this selective permeability for $K^+$, every $K^+$ ion that exits the cell leaves one extra negative charge (an extra $A^-$) on the inside of the cell and adds one extra positive charge on the outside of the cell. This separation of charges across the membrane creates a negative membrane potential.

If a negative membrane potential was not established as $K^+$ leaves the cell, then $K^+$ ions would continue to exit until the extracellular concentration of $K^+$ was equal to the intracellular concentration of $K^+$. However, the establishment of a voltage difference (attributable to the charged nature of the $K^+$ and $A^-$ ions) creates an **electrostatic force** that eventually prevents net $K^+$ efflux (Fig. 5-3B). Thus, the electrical gradient ($V_m$) and chemical gradient "pull" the $K^+$ ions in opposite directions; the electrical gradient favors an inward flow of $K^+$ ions, while the chemical gradient favors an outward flow of

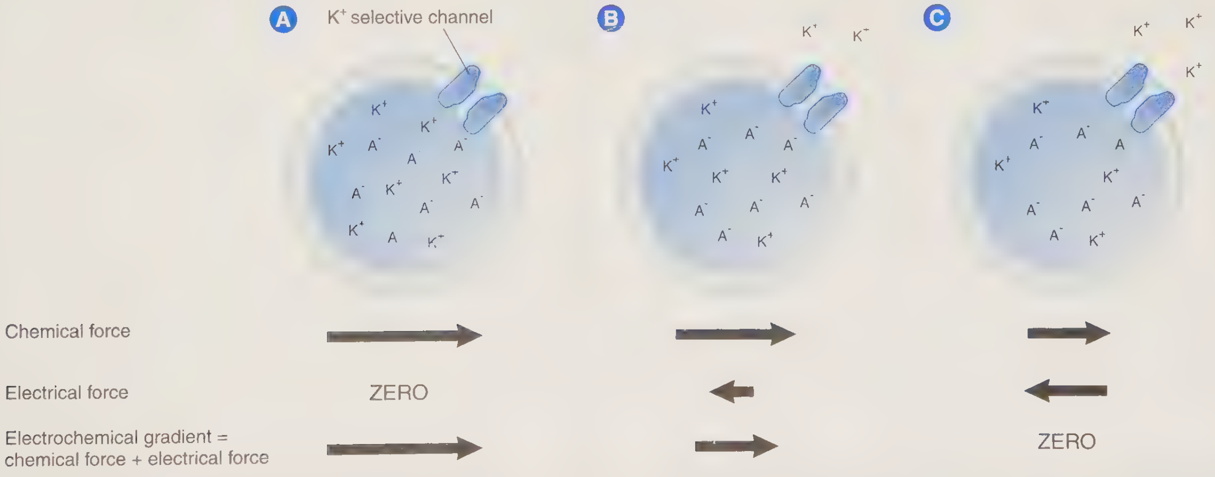


**Figure 5-3. Electrochemical Basis of the Resting Membrane Potential.** **A.** Consider a prototypical cell that initially has equal concentrations of intracellular potassium ions ($K^+$) and impermeant anions ($A^-$). Assume further that ions can exit the cell only via a single $K^+$-selective channel. In this case, there is a strong chemical gradient for both $K^+$ and $A^-$ to exit the cell, but there is no electrical force favoring ion flow because the electrical sum of the intracellular charges is zero. **B.** $K^-$ begins to exit the cell through the $K^+$-selective channel, but A remains inside the cell because it has no exit route. Therefore, the $K^+$ chemical gradient across the membrane becomes smaller. As $K^+$ exits the cell, however, the net negative charge from the $A^-$ remaining inside the cell produces a negative membrane potential that exerts an electrical force disfavoring $K^+$ efflux. This force is opposite in direction to that of the chemical gradient; as a result, the total electrochemical gradient (the sum of the chemical force and the electrical force) is less than the chemical gradient alone. **C.** When the electrical gradient is equal and opposite to the chemical gradient, the system is in equilibrium, and no net ion flow occurs. The voltage resulting from the separation of charges at equilibrium is referred to as the Nernst potential.

$K^+$ ions. These forces summate to create an **electrochemical gradient.** *The transmembrane electrochemical gradient is the net driving force for ion movements through channels in biological membranes.*

As a result of the electrochemical gradient, the extracellular concentration of $K^+$ does not equilibrate with the intracellular concentration. Instead, an equilibrium is established in which the electrostatic force "pulling" $K^+$ back into the cell is balanced exactly by the chemical gradient favoring $K^+$ efflux. The potential at which this equilibrium occurs, for any permeant ion X, is a function of the charge of the ion (z), the temperature (T), and the intracellular and extracellular concentrations of the ion. This relationship is expressed as the **Nernst equation**:

$$V_x = V_{in} - V_{out} = \frac{RT}{zF} \ln \frac{[X]_{out}}{[X]_{in}} \qquad \text{Equation 5-2}$$

where $V_x$ is the transmembrane potential that a membrane selectively permeable to ion $X$ would reach at equilibrium (i.e., the **Nernst potential** for that ion), $V_{in} - V_{out}$ is the transmembrane voltage difference, $RT/zF$ is a constant for a given temperature and charge (this number simplifies to 26.7 mV for a charge of +1 at a temperature of 37°C), and $[X]_{out}$ and $[X]_{in}$ are the extracellular and intracellular concentrations, respectively, of ion $X$. The electrochemical driving force on ion $X$ is equal to the difference between the actual membrane potential and the Nernst potential for that ion, $V_m - V_x$.

The third requirement of the resting membrane potential, that active and passive ion pumps maintain ion gradients across the membrane, determines the concentration of ions inside and outside the cell. Numerous pumps play an important physiologic role in maintaining ion gradients; these include the ATP-dependent $Na^+/K^+$ pump (which extrudes three $Na^+$ ions for every 2 $K^+$ ions that enter the cell) and the $Na^+/Ca^{2+}$ exchanger (which extrudes one $Ca^{2+}$ for every three $Na^+$ ions that enter the cell). The coordinated action of these pumps closely regulates the intracellular and extracellular concentrations of all the important cations and anions. By knowing the values of these ion concentrations, it is possible to calculate the Nernst potentials for these cations and anions at physiologic temperature and, hence, the value of the transmembrane potential at which the net driving force for a particular ion vanishes (Table 5-1).

The differences between the extracellular and intracellular concentrations of the four major ions are attributable to variations in the extent of transport for each ion mediated by pumps and exchangers in the plasma membrane and to variations in membrane permeability mediated by channels selective for each ionic species. Relative ionic permeabilities of the neuronal membrane at rest, ranked in decreasing order, are $K^+ >> Cl^- > Na^+ >> Ca^{2+}$. Because $K^+$ is the most permeant ion under resting conditions, the resting membrane potential most closely approximates the Nernst potential for $K^+$ (about –90 mV). In reality, the weak permeability of other ionic species raises the potential above that for $K^+$. Thus, although $K^+$ is the ion of predominant permeability, the other ions and the so-called "electrogenic" pumps that produce net flows of current also contribute to the overall resting potential. At the steady state that describes the true cellular resting potential (Fig. 5-4), the membrane potential $V_m$ does not equal the Nernst potential for any of the individual ions, and each ion species experiences a net electrochemical force. In other words, $(V_m - V_{ion})$ is nonzero, and small ion fluxes occur. The algebraic sum of these inward and outward currents is small and is just balanced by currents from active, electrogenic pumps, so there is no *net* current across the resting membrane. It has been estimated that up to 25% of all cellular energy is expended in maintaining ion gradients across cellular membranes.

## THE GOLDMAN EQUATION

The example shown in Figure 5-3 addresses a situation where only one ion species flows across the plasma membrane. In reality, cells possess a number of different ion-selective channels, all of which contribute to the overall resting membrane voltage. When the resting potential is determined by two or more species of ions, the influence of each species is determined by its concentrations inside and outside the cell and by the permeability of the membrane to that ion. Quantitatively, this relationship is expressed as the **Goldman-Hodgkin–Katz Equation**:

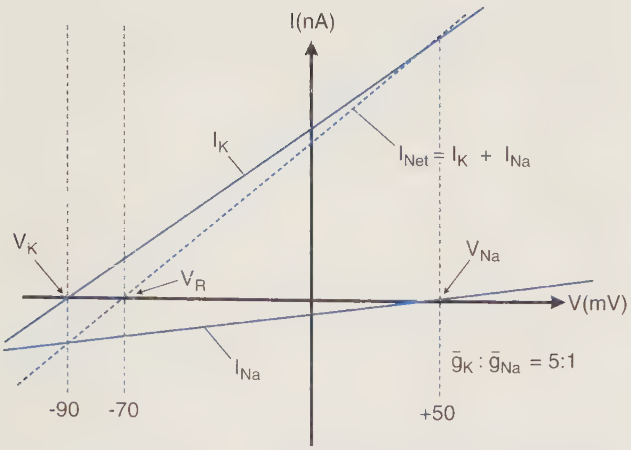
$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \qquad \text{Equation 5-3}$$

where $P_x$ is the membrane permeability of a given ion. ($P_x$ is expressed as a fraction, with a value of 1 indicating maximum permeability.) Essentially, this expression says that the greater the concentration of a particular ion and the greater its membrane permeability, the greater its role in determining the membrane potential. In the extreme case, when the permeability of one ion is exclusively dominant, the Goldman

**TABLE 5-1** Nernst Equilibrium Potentials for Major Ions

| ION | EXTRACELLULAR CONCENTRATION | INTRACELLULAR CONCENTRATION | NERNST EQUATION FOR ION | NERNST POTENTIAL FOR ION |
|---|---|---|---|---|
| $Na^+$ | 145 mM | 15 mM | 26.7 ln (145/15) | $V_{Na^+} = +61$ mV |
| $K^+$ | 4 mM | 140 mM | 26.7 ln (4/140) | $V_{K^+} = -95$ mV |
| $Cl^-$ | 122 mM | 4.2 mM | −26.7 ln (122/4.2) | $V_{Cl^-} = -90$ mV |
| $Ca^{2+}$ | 1.5 mM | $\approx 1 \times 10^{-5}$ mM | $26.7/2 \ln (1.5/1 \times 10^{-5})$ | $V_{Ca^{2+}} = +159$ mV |

The calculated values of the Nernst potential are typical of mammalian skeletal muscle. Many human cells have similar transmembrane ion gradients.



$I_K$ - $K^+$ current
$V_K$ - $K^+$ Nernst potential
$\bar{g}_K$ - $K^+$ conductance
$I_{Na}$ - $Na^+$ current
$V_{Na}$ - $Na^+$ Nernst potential
$\bar{g}_{Na}$ - $Na^+$ conductance
$V_R$ - Resting membrane potential
$I_{Net}$ - Net current

**Figure 5-4. Relative Contribution of K⁺ and Na⁺ to the Resting Membrane Potential.** The relative membrane permeabilities of $K^+$, $Na^+$, and other ions, and the Nernst (electrochemical equilibrium) potentials of these ions, together determine the resting membrane potential. In the example shown, the conductance of $K^+$ is about five times the conductance of $Na^+$ (shown by the slopes of the I vs. V lines for $I_K$ and $I_{Na}$, respectively). That is, the membrane is five times more permeable to $K^+$ than to $Na^+$. The $K^+$ current is described by $I_K$ [$I_K = \bar{g}_K(V-V_K)$], while the $Na^+$ current is described by $I_{Na}$ [$I_{Na} = \bar{g}_{Na}(V-V_{Na})$]. (In this example, $\bar{g}_K$ and $\bar{g}_{Na}$ are constant conductances over all voltages.) $I_{Net}$, the net membrane current, is the sum of these two currents ($I_{Net} = I_K + I_{Na}$). The "resting" membrane potential ($V_R$) is the value of V at which $I_{Net}$ equals zero. Note in the example that $V_R$ is close to, but greater than, $V_K$. This is because, although $K^+$ is the primary determinant of the resting potential, the minor $Na^+$ current depolarizes $V_R$ to a value less negative than $V_K$.

equation reverts to the Nernst equation for that ion. For example, if $P_K >> P_{Cl}, P_{Na}$, the equation becomes

$$V_m = \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i}$$

Alternatively, if $P_{Na}$ greatly exceeds $P_K, P_{Cl}$, then $V_m \sim V_{Na}$, and the membrane is strongly depolarized. This important concept links changes in ion channel permeability to changes in membrane potential. *Whenever an ion-selective channel opens, the membrane tends to approach the Nernst potential for that ion.* The relative contribution of a given channel to the overall membrane potential depends on the extent of ion flow through that channel (represented by the permeability). Time-dependent changes in the membrane permeabilities of $Na^+$ and $K^+$ (and, in cardiac cells, $Ca^{2+}$) ions account for the major distinguishing feature of electrically excitable tissues—the action potential.

## THE ACTION POTENTIAL

According to Ohm's law, passage of a small amount of current across a cell membrane causes the voltage across the membrane to change, reaching a new steady-state value that is determined by the membrane's resistance (see above). The time course of this voltage change is determined by the product of the resistance $r_m$ and the capacitance $C_m$ of the membrane, with a rate constant equal to $[r_m \cdot C_m]^{-1}$. (The membrane's capacitance results from having an insulator, the hydrocarbon core of the phospholipids in the membrane, between two conductors, the ionic solutions on either side of the membrane [see Fig. 5-2]. Capacitors store charge at both surfaces and require time to change this charge.) If the stimulated potential change is less than the **threshold** value, then the membrane voltage changes smoothly and returns to its resting value when the current is turned off (Fig. 5-5A). On the other hand, if the membrane voltage changes positively by more than the threshold value, then a more dramatic event occurs; the membrane voltage rises to a value of approximately +50 mV and then drops to its resting value of approximately –80 mV (Fig. 5-5B). This "suprathreshold" event is known as the **action potential** (AP). An action potential cannot be produced by even a large hyperpolarizing stimulus; in this case, the membrane voltage response remains continuous and scaled to the external current, depending only on the resting membrane's resistance and capacitance (Fig. 5-5C).

In most neurons, the balance between voltage-gated $Na^+$ and $K^+$ channels regulates the action potential. (In some cardiac cells, voltage-gated $Ca^{2+}$ cells are also involved in AP regulation; see Chapter 17.) Voltage-gated $Na^+$ channels conduct an inward current that depolarizes the cell at the beginning of the AP. Voltage-gated $K^+$ channels conduct an outward current that repolarizes the cell at the end of the AP, in preparation for the next excitatory event. Figure 5-6 shows the current-voltage (I–V) relationships for the voltage-gated $Na^+$ channel and the "resting" $K^+$ channel. The total $Na^+$ conductance of a membrane is the product of the conductance of a single open $Na^+$ channel, the total number of $Na^+$ channels, and the probability that an individual $Na^+$ channel is open, $P_o$. Key to the excitability of a membrane is the voltage-dependence of $P_o$, shown in Figure 5-6A. Rapid membrane depolarizations to –50 mV or above cause $Na^+$ channels to open, with a probability that increases to 1.0, the maximum value, at about zero millivolts. The open channel probability represents the fraction of all $Na^+$ channels that open in response to a single voltage step. For example, at very negative potentials (e.g., –85 mV) essentially no channels are open; as the AP depolarizes the membrane through 0 mV most or all channels open; and fast depolarizations to –30 mV open about half of the channels.

Recall that ionic current is the product of the ionic conductance ($g$) and a potential difference. For ions, the potential difference is the same as the electrochemical driving force, $V_m - V_x$, where $V_x$ is the Nernst potential for the specified ion. For example, for $Na^+$ current:

$$I_{Na} = g_{Na}(V_m - V_{Na})$$

or

$$I_{Na} = \bar{g}_{Na}P_o(V_m - V_{Na}) \qquad \text{Equation 5-4}$$

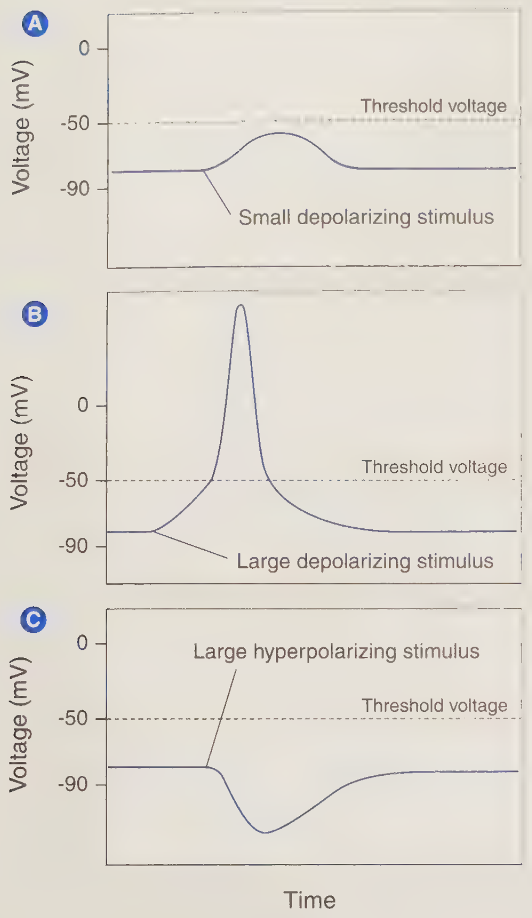


**Figure 5-5. The Action Potential.** **A.** In the example shown, a resting cell has a membrane potential of approximately –80 mV. If a small depolarizing stimulus is applied to the cell (for example, the opening of voltage-gated $Ca^{2+}$ channels), the membrane slowly depolarizes in response to the increased permeability of the membrane to $Ca^{2+}$. Once the stimulus ends (the $Ca^{2+}$ channels close), the membrane returns to its resting potential. The time course of the voltage change is determined by the membrane capacitance (see Fig. 5-2). **B.** If a larger depolarizing stimulus is applied to the cell, so that the membrane potential exceeds its "threshold" voltage, the membrane rapidly depolarizes to about +50 mV and then returns to its resting potential. This event is known as an **action potential;** its magnitude, time course, and shape are determined by voltage-gated $Na^+$ and $K^+$ channels that open in response to membrane depolarization. **C.** In comparison, application of a hyperpolarizing stimulus to a cell does not generate an action potential, regardless of the magnitude of hyperpolarization.

Here, $\bar{g}_{Na}$ is the $Na^+$ conductance of the membrane when all $Na^+$ channels are open, and $P_o$ is, as above, the probability of the $Na^+$ channels being open. The graphic illustration of this equation is shown in Figure 5-6B, where the $Na^+$ current for a "fully activated" membrane is described by the straight line that passes with positive slope through $V_{Na}$. If there were no voltage-dependence to the $Na^+$ conductance (i.e., if $g_{Na}$ were always equal to $\bar{g}_{Na}$), this line would extend throughout the negative voltage range, as shown by its dashed line extrapolation. However, the voltage-dependence of $P_o$ (Fig. 5-6A)

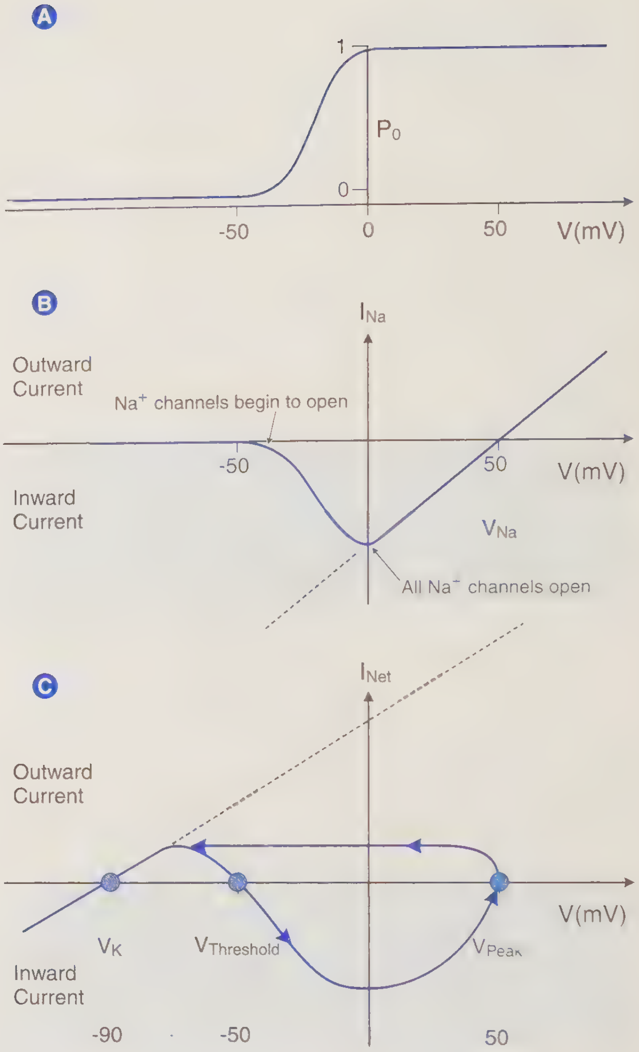


**Figure 5-6. Voltage Dependence of Channel Activity.** **A.** $P_o$, the probability that an individual voltage-gated $Na^+$ channel will be open, is a function of the membrane voltage (V). At voltages less than –50 mV, there is a very low probability that a voltage-gated sodium channel will be open. At voltages greater than –50 mV, this probability begins to increase and approaches 1 (i.e., a 100% chance of being open) at 0 mV. These probabilities are also generalizable to a population of voltage-gated channels, so that virtually 100% of voltage-gated $Na^+$ channels in the membrane will be open at 0 mV. **B.** The $Na^+$ current across a membrane is a function of the voltage-dependence of the $Na^+$ channels that carry the current. At voltages below –50 mV, the $Na^+$ current is zero. As the voltage increases above –50 mV, $Na^+$ channels begin to open, and there is an increasingly inward (negative) $Na^+$ current. The maximum inward $Na^+$ flux is reached at 0 mV, when all the channels are open. As the voltage continues to increase above 0 mV, the $Na^+$ current is still inward but decreasing because inward flow of the positively charged $Na^+$ ions is opposed by the increasingly positive intracellular potential. The $Na^+$ current at $V_{Na}$ (the Nernst potential for $Na^+$) is zero because at this voltage the electrical and chemical gradients for $Na^+$ ion flow are balanced. At voltages greater than $V_{Na}$, the $Na^+$ current is outward (positive). The dashed line indicates the relationship that would exist between $Na^+$ current and voltage if the open probability of the $Na^+$ channels were not voltage-dependent. **C.** The summation of plasma membrane $Na^+$ currents and $K^+$ currents demonstrates three key transition points in the I–V graph (denoted by blue circles) at which the net current is zero. The first of these points occurs at a membrane potential of –90 mV, where $V = V_K$. At this voltage, a

causes the real Na$^+$ conductance $g_{Na}$ to be voltage-dependent, resulting in deviation of the real $I_{Na}$ from this theoretical "fully activated" maximum condition. Thus, increasing depolarizations from rest (caused, for example, by an applied stimulus) result in inward Na$^+$ currents that first become larger as more channels open and then become smaller as $V_m$ approaches $V_{Na}$ (Fig. 5-6B).

Potassium channels conduct outward currents that oppose the depolarizing actions of inward Na$^+$ currents. Although there are many types of K$^+$ channels with diverse "gating" properties, to appreciate the role of K$^+$ channels in excitability only two types need to be considered. These two K$^+$ channel types include the voltage-independent "leak" channels and the voltage-gated "delayed rectifier" channels. **Leak channels** are the K$^+$ channels that contribute to the resting membrane potential by remaining open throughout the negative range of membrane potentials. The K$^+$ current that flows through these channels is shown by the dashed line in Figure 5-6C; for these channels, K$^+$ current is outward for all $V_m > V_K$.

The sum of $I_{Na}$ and $I_K$ (leak) is represented by the solid line in Figure 5-6C. Three important points on this line, at which the net ionic current is zero, define three critical aspects of the action potential. First, at rest, $V_m \approx V_K$. Under this condition, small membrane depolarizations caused by "external" influences (e.g., by sensory inputs) result in net outward currents that repolarize the membrane back to rest when the external stimulus ends. Second, at $V_m = V_T$, the outward potassium currents are matched by inward sodium currents, and the net current is also zero. Under this condition, however, even a small further depolarization results in a net inward current that further depolarizes the membrane, which leads to a larger inward current and further membrane depolarization. This positive feedback loop constitutes the **rising phase** of the action potential. Thus, the action potential occurs in response to any rapid depolarization beyond $V_T$, which is defined as the **threshold potential.** Third, $V_p$ is the potential at the peak of the action potential. Once $V_m$ reaches this maximum depolarization, the net current switches sign from inward to outward and, consequently, the membrane begins to be repolarized.

Voltage-gated **(delayed rectifier)** K$^+$ channels contribute to the initial, rapid repolarization phase of the AP. Although depolarization opens these channels, they open and close more slowly than do Na$^+$ channels in response to depolarization. Therefore, inward Na$^+$ current dominates the early depolarization phase of the AP, and outward K$^+$ current dominates the later repolarization phase (Fig. 5-7). This is why the action potential is characterized by an initial rapid depolarization (caused by fast inward Na$^+$ current), followed by a prolonged repolarization (caused by slower and more sustained outward K$^+$ current).

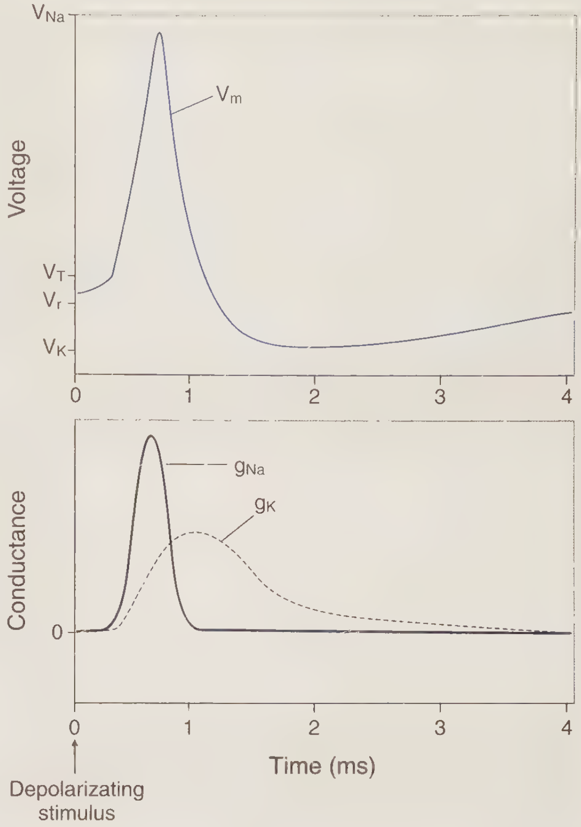


**Figure 5-7. Time Course of Voltage-Dependent Na$^+$ and K$^+$ Conductances.** During the course of an action potential, the transmembrane voltage ($V_m$) first increases rapidly from $V_T$ toward $V_{Na}$, then decreases below $V_T$ and more slowly approaches $V_K$. The shape and duration of the action potential can be explained by the differential time courses of the voltage-dependent Na$^+$ and K$^+$ currents. In response to a depolarizing stimulus, the Na$^+$ conductance ($g_{Na}$) increases rapidly because of the rapid opening of voltage-gated Na$^+$ channels, then decreases because of Na$^+$ channel inactivation and, later because $V_m$ falls below $V_T$. The K$^+$ conductance ($g_K$) increases concurrently with $g_{Na}$, but takes a longer time to reach its maximum conductance because there is a slower rate constant for opening of voltage-dependent K$^+$ channels. Eventually, $g_K$ is greater than $g_{Na}$, and the membrane repolarizes. ($V_{Na}$, $V_K$, Nernst potentials for Na$^+$ and K$^+$, respectively. $V_r$, resting membrane potential. $V_T$, threshold potential for action potential firing.)

The limited duration of Na$^+$ channel opening in response to depolarization is the final feature that determines membrane excitability. After opening in response to rapid membrane depolarization, Na$^+$ channels enter a closed state in which they are **inactivated** (i.e., prevented from subsequent opening). Recovery from inactivation occurs only when the membrane is repolarized, whereupon the Na$^+$ channels return to the closed, resting state from which they can open in response to a stimulus. This inactivation of Na$^+$ conductance, combined with the slowly decaying voltage-gated K$^+$ conductance, produces dynamic changes in membrane excitability. Following just one AP, fewer Na$^+$ channels are available to open (i.e., $\bar{g}_{Na}$ is temporarily smaller), more K$^+$ channels

small increase in potential (i.e., a small depolarization) results in a net outward (positive) K$^+$ current that brings the membrane potential back toward $V_K$. The second point occurs at $V_{Threshold}$, the threshold voltage ($V_T$). At this voltage, further depolarization results in the opening of voltage-dependent Na$^+$ channels and a net negative (inward) current, known as the action potential. The third point occurs at $V_{Peak}$, the peak voltage ($V_P$). At this voltage, the transition occurs from a net negative current to a net positive current caused by the opening of voltage-dependent K$^+$ channels. This results in the return of the membrane potential toward $V_K$; i.e., in membrane repolarization. The dashed line indicates the I–V relationship for the "leak" (voltage-independent) K$^+$ channels.

are open (i.e., $g_K$ is larger), the corresponding ionic currents are changed, and $V_T$ is more positive than it was before the AP. An excitable membrane is in its so-called **refractory state** during this period, which lasts from just after the AP until the conditions of fast $g_{Na}$ inactivation and slow $g_k$ activation have returned to their resting values. Slow depolarizing stimuli will fail to induce an action potential, even when the membrane reaches the $V_T$ defined by a rapid depolarizing stimulus, because of the accumulated inactivation of $Na^+$ channels during the stimulus. The inactivation property of $Na^+$ channels is important in the concept of use-dependent block, as discussed in Chapter 9, Local Anesthetic Pharmacology, and Chapter 17, Pharmacology of Cardiac Rhythm.

## PHARMACOLOGY OF ION CHANNELS

Many drugs act directly on ion channels to produce changes in membrane excitability. For example, local anesthetics are injected locally at high concentrations to block $Na^+$ channels in peripheral and spinal neurons; this $Na^+$ channel block inhibits action potential propagation and prevents sensory transmission (e.g., pain) by these nerves (see Chapter 9). At much lower concentrations, these and structurally similar antiarrhythmic drugs act systemically to suppress abnormal action potentials in the heart and to treat neuropathic pain and some forms of myotonia (see Chapter 17). Drugs that block $K^+$ channels are used to treat certain types of cardiac arrhythmias, and may be used in the future to overcome nerve conduction deficits secondary to demyelinating conditions such as multiple sclerosis or spinal cord injury. Calcium channels are blocked directly by some drugs used in the treatment of hypertension; such drugs act by relaxing vascular smooth muscle and lowering systemic vascular resistance. Some heart conditions are also treated by selective blockers of cardiac $Ca^{2+}$ channels (see Chapter 20, Pharmacology of Vascular Tone). Highly potent and selective blockers of a certain class of neuronal $Ca^{2+}$ channel have been purified from the venom of a marine snail (*Conus* sp.), and administered into the spinal fluid to treat severe cases of neuropathic pain. Ion channels may also be modified by drugs indirectly, through pharmacologic modulation of the receptors that regulate the channels, as described below.

## ELECTROCHEMICAL TRANSMISSION

One result of a neuronal action potential is the release of neurotransmitters, which are small molecules or peptides that can stimulate cell surface receptors on muscle cells, endocrine cells, or neurons. Transmitters may be released into the circulation, from which they can act on distant organs, or may diffuse only a very short distance to act on juxtaposed target cells at a specialized connection called a synapse. Synaptic transmission thus integrates electrical signals (voltage changes in the plasma membrane of the presynaptic cell) with chemical signals (release of neurotransmitter by the presynaptic cell and subsequent binding of the transmitter to receptors in the membrane of the postsynaptic cell). For this reason, synaptic transmission is often referred to as **electrochemical transmission.**

There is a general sequence of processes that are essential for electrochemical transmission (Fig. 5-8):

1. Transmitter is synthesized by special enzymes and stored in vesicles.
2. The action potential is propagated over the presynaptic membrane.

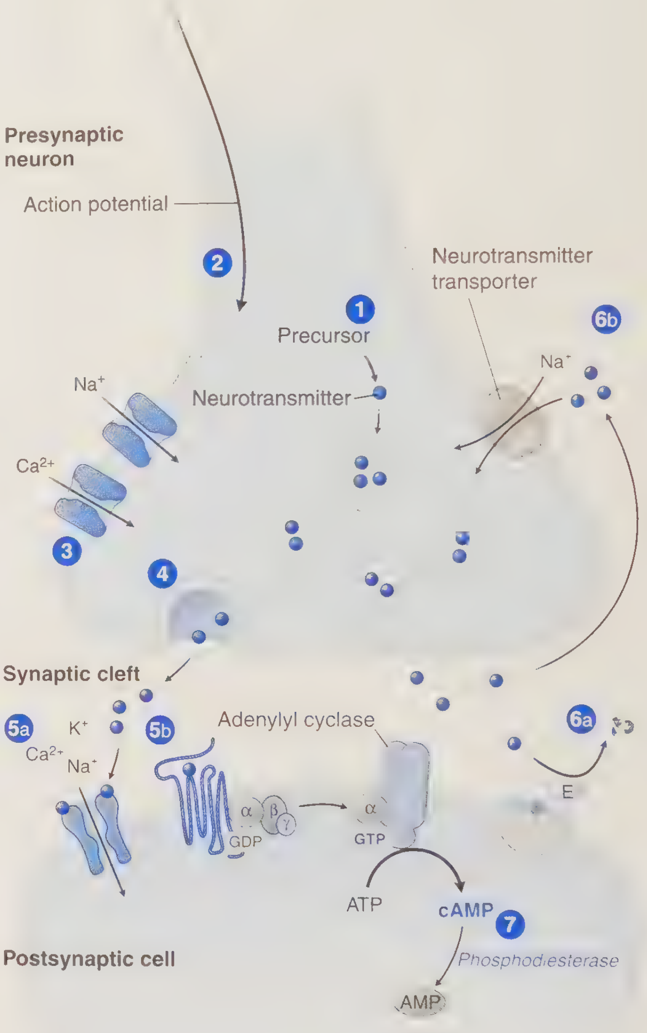


**Figure 5-8. Steps in Synaptic Transmission.** Synaptic transmission can be divided into seven steps that couple electrical depolarization of the presynaptic cell to chemical signaling between the presynaptic and postsynaptic cells. **1.** Neuron synthesizes neurotransmitter from precursor, and stores the transmitter in vesicles. **2.** An action potential traveling down the neuron depolarizes the cell. **3.** Membrane depolarization activates voltage-dependent $Ca^{2+}$ channels, allowing $Ca^{2+}$ entry into the cell. **4.** The increased cytosolic $Ca^{2+}$ enables vesicle fusion with the plasma membrane of the presynaptic neuron, with subsequent release of neurotransmitter into the synaptic cleft. **5.** Neurotransmitter diffuses across the synaptic cleft and binds to one of two types of postsynaptic receptors. **5a.** Neurotransmitter binding to ionotropic receptors causes channel opening and changes in the membrane permeability to ions. This may also result in a change

3. Depolarization of the presynaptic terminal opens the voltage-dependent $Ca^{2+}$ channels and results in calcium influx.
4. The increased calcium in the presynaptic terminal causes vesicles to fuse with the presynaptic membrane, which in turn causes transmitter to be released into the synaptic cleft.
5. Released transmitter can bind to two classes of receptors on the postsynaptic membrane.
    a. Binding of transmitter to ligand-gated ionotropic receptors in the postsynaptic membrane directly opens ion channels, permitting ion flow that leads rapidly to **excitatory** or **inhibitory postsynaptic potentials.**
    b. Binding of transmitter (ligand) to **metabotropic** (e.g., G protein-coupled) receptors results in an intracellular second messenger signaling cascade. These signaling events can also lead to changes in the postsynaptic potential, although their time course is slower (generally seconds to minutes). (Some transmitters may also bind to **"autoreceptors"** on the presynaptic membrane and thereby regulate transmitter release.)
6. Excitatory postsynaptic potentials (EPSPs) or inhibitory postsynaptic potentials (IPSPs) extend passively (i.e., without generating an action potential) over the postsynaptic cell. A large number of EPSPs can summate to cause the postsynaptic membrane to exceed threshold voltage ($V_T$). If this occurs, a new action potential may be generated in the postsynaptic cell. (This process is not shown in Fig 5-8.)
7. Stimulation of the postsynaptic cell(s) by transmitter is terminated by removal of the transmitter. This removal can be attributable to the following:
    a. Degradation of free transmitter molecules by enzymes in the synaptic cleft, and/or
    b. Reuptake of the transmitter into the presynaptic terminal (or by surrounding glial cells), which both stops synaptic action and ultimately allows transmitter to be recycled into vesicles.
8. For G protein-coupled metabotropic receptors in the postsynaptic cell, termination of the response to a transmitter stimulus is also dependent on intracellular enzymes that inactivate second messengers (e.g., phosphodiesterases that convert active cAMP to inactive AMP).

The classically described chemical synapse is that of the neuromuscular junction. At this junction, terminal branches of the motor axon lie in a synaptic trough on the surface of the muscle cells. When the neuron fires, acetylcholine (ACh) is released from the motor neuron terminals, causing a postsynaptic depolarization of the muscle cell, the end plate potential, that nearly always leads to a single muscle contraction or "twitch." The released ACh diffuses across the **synaptic cleft** to bind to ligand-gated ionotropic receptors located on the postsynaptic muscle. This binding of ACh to its receptors causes a transient increase in the probability of opening of the receptor-associated ion channels, which have a pore that is equally permeable to $Na^+$ and $K^+$. As a result, these channels have a **reversal potential** (i.e., a potential at which there is no net current flowing through the channel) of approximately 0 mV (the average of the individual $Na^+$ and $K^+$ Nernst potentials). The net inward current passing through these open channels depolarizes the muscle membrane. Although this particular end-plate potential is always sufficiently large to stimulate an action potential in the muscle, its magnitude of depolarization is the exception. Most neuronal "excitatory postsynaptic potentials" are only a few millivolts in amplitude, and many of them must occur together, within a short time (~10 ms) and at closely spaced synapses for the postsynaptic depolarization to reach the threshold value for firing of an action potential.

The following discussion highlights points in the basic steps of neurotransmission that can be modified by pharmacologic agents.

## SYNAPTIC VESICLE REGULATION

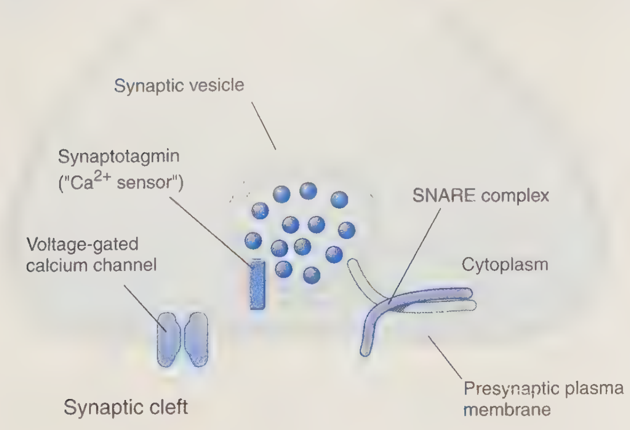
Nerve terminals contain two types of secretory vesicles: small, clear-core synaptic vesicles and large, dense-core vesicles. The clear synaptic vesicles store and secrete small organic neurotransmitters, such as acetylcholine, GABA, glycine, and glutamate. Large dense-core vesicles, on the other hand, are more likely to contain peptide or amine neurotransmitters. The larger vesicles are more similar to the secretory granules of endocrine cells because their release is not limited to "active zones" on the presynaptic cell and is more likely to follow trains of impulses (continuous or rhythmic stimulation) than single action potentials. Hence, the smaller synaptic vesicles are involved in rapid chemical transmission, while the larger dense-core vesicles are implicated in slow, modulatory, or distant signaling.

Over the past several years, some of the proteins that control synaptic vesicle trafficking have been identified. **Synapsin,** the first synaptic vesicle protein to be identified, is a peripheral membrane protein that binds to both synaptic vesicles and actin. Investigators have proposed that this protein physically links vesicles to the cytoplasmic actin matrix at nerve terminals. In addition, because synapsin is a major endogenous substrate of protein kinases regulated by cAMP and $Ca^{2+}$/calmodulin, it is thought that these second messengers have a crucial regulatory role in neurotransmitter release by controlling the availability of synaptic vesicles for $Ca^{2+}$-dependent exocytosis. **Synaptobrevin** is a small protein anchored to the vesicle membrane by a hydrophobic C-terminal region. It is a transmembrane protein, one of several proteins collectively called **SNAREs** (soluble N-ethylmaleimide-sensitive factor attachment protein receptors), that are essential for both $Ca^{2+}$-regulated and $Ca^{2+}$-independent vesicle exocytosis (Fig. 5-9). Certain neurotoxins that act as highly selective inhibitors of synaptic vesicle exocytosis, such as tetanus toxin and botulinum toxin (see Chapter 7), seem to work by selectively cleaving synaptobrevin, implicating this protein in the fusion step of exocytosis. Synapsin, synaptobrevin, and the

←

in the postsynaptic membrane potential. **5b.** Neurotransmitter binding to metabotropic receptors on the postsynaptic cell activates intracellular signaling cascades, including G protein activation and formation of cAMP by adenylyl cyclase. These signaling cascades can, in turn, activate other ion-selective channels. **6.** Signal termination is accomplished by removal of transmitter from the synaptic cleft. **6a.** Transmitter can be degraded by enzymes (E) present in the synaptic cleft. **6b.** Alternatively, transmitter can be returned to the presynaptic cell by reuptake transporters. **7.** In addition, signal termination can be accomplished by enzymes (such as phosphodiesterase) that degrade postsynaptic intracellular signaling molecules (such as cAMP).

**A** Synaptic vesicle primed for release of neurotransmitter


Synaptic vesicle
Synaptotagmin ("$Ca^{2+}$ sensor")
Voltage-gated calcium channel
SNARE complex
Cytoplasm
Presynaptic plasma membrane
Synaptic cleft

**B** Local $Ca^{2+}$ transient triggers release of neurotransmitter into synaptic cleft

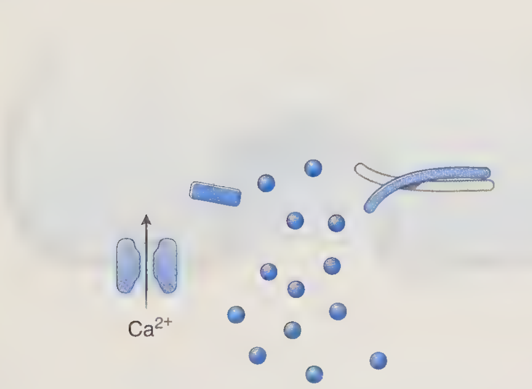


**Figure 5-9. Detailed Mechanism of Neurotransmitter Release.** **A.** Synaptic vesicles are tethered close to the plasma membrane of the presynaptic neuron by several protein–protein interactions. The most important of these interactions involve SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins in the vesicle membrane and the plasma membrane. Voltage-gated calcium channels in the plasma membrane are located close to these SNARE complexes to facilitate the sensing of $Ca^{2+}$ entry by synaptotagmin in the vesicle membrane. **B.** In response to an action potential, voltage-gated calcium channels open, allowing entry of extracellular $Ca^{2+}$ into the cell. The increase in intracellular $Ca^{2+}$ triggers fusion of the vesicle membrane with the plasma membrane, releasing neurotransmitter molecules into the synaptic cleft.

many other proteins involved in the various steps of neurotransmitter release may, in the future, provide biochemical targets for pharmacologic control of synaptic transmission.

## POSTSYNAPTIC RECEPTORS

By far the largest number of neuropharmaceuticals is directed toward postsynaptic receptors. Ionotropic receptors, such as nicotinic receptors for acetylcholine (such as that at the neuromuscular junction) and "A" type receptors for $\gamma$-aminobutyric acid (the major inhibitory neurotransmitter in the CNS), are almost always composed of 4–5 subunits that aggregate in the membrane to form a ligand-gated channel. Binding to the receptor of one (or sometimes two) ligand (called "agonist") molecules leads to allosteric conformational changes that open the pore. The subunits composing the same functional receptor often differ among different tissues and, as a consequence, the detailed molecular pharmacology of the receptors is tissue dependent. For example, although acetylcholine is the endogenous transmitter for all nicotinic cholinergic receptors, a number of synthetic agonists (or antagonists) selectively activate (or inhibit) these receptors in skeletal muscle, autonomic ganglia, or the CNS (see Chapter 7).

Receptors within different regions of the same organ can also vary in their subunit composition. In the brain, for example, different $GABA_A$ receptor subunits compose different forms of the functional receptor in the cerebellum, cerebral cortex, and hippocampus. Each of these forms has a selective pharmacology, so that subtype-selective antagonists (e.g., general anesthetics) produce a unique set of behavioral and neurological changes during the induction of general anesthesia (see Chapter 14, General Anesthetic Pharmacology).

Metabotropic receptors are similarly diverse. Although most are G protein-coupled receptors (GPCRs) with seven membrane-spanning alpha-helical segments, the extracellular and cytoplasmic domains of these receptors are highly differentiated. This differentiation has allowed the identification and development of a great range of agonists (and antagonists) that activate (and inhibit) the GPCRs, and the identification of a variety of G proteins through which the GPCRs regulate cellular processes. Agonist and antagonist binding domains of GPCRs may be located on extracellular loops, on sites formed by a combination of extracellular loops and segments of transmembrane helices, and even in cavities formed by several helices within the membrane. Increasingly sophisticated access to this rich variety of structural motifs and binding domains suggests that neurotransmitter receptors represent especially promising targets for the development of selective drugs.

## TRANSMITTER METABOLISM AND REUPTAKE

Altering the metabolism of neurotransmitters provides useful mechanisms of pharmacologic intervention at the synapse. The two major types of intervention include inhibition of neurotransmitter degradation and antagonism of neurotransmitter reuptake. An example of a class of degradation inhibitors is the drugs that decrease the activity of acetylcholinesterase, the enzyme responsible for degrading acetylcholine. These drugs are often used in the treatment of myasthenia gravis (see Chapter 7).

Of growing pharmacologic importance are the transporters that facilitate neurotransmitter reuptake from the synaptic cleft into the presynaptic cell. Because these transporters are crucial for the termination of synaptic transmission, their manipulation may have profound effects in the nervous system. For example, the psychotropic effects of cocaine derive from this drug's inhibition of dopamine and norepinephrine reuptake in the brain, and the therapeutic benefit of such antidepressants as fluoxetine result from their inhibition of serotonin-selective reuptake (see Chapter 12, Pharmacology of Affective Disorders). Because reuptake transporters have specific structural requirements for their endogenous substrates, it is hoped that structurally selective drugs can be designed to target specific transporter subtypes with minimal effects on other transporters.

## *Conclusion and Future Directions*

Cellular excitability is a crucial component of intercellular communication. The fundamental basis for cellular excitability lies in the electrochemical gradients that are established by ion pumps across the plasma membrane's lipid bilayer. Ion-selective channels enable cellular membranes to regulate selectively the permeability of the membrane to different ionic species, allowing a change in membrane voltage to be tied to a chemical stimulus or response. The action potential, a special type of stereotyped response found in excitable cells, is made possible by the voltage-dependent properties of $Na^+$ and $K^+$ channels.

The action potential is an important initiating event for electrochemical transmission, a process that translates electrical depolarization into a chemical stimulus. This chemical stimulus—in most cases, the release of a neurotransmitter—diffuses across the synaptic cleft separating the presynaptic and postsynaptic cells. Binding of the neurotransmitter to its postsynaptic receptors initiates changes in ion permeability in the target cell membrane, which can lead to membrane depolarization and changes in cellular signaling.

The basic processes of electrochemical transmission provide the substrate for pharmacologic modulation of cellular excitation and communication, topics that are addressed in more detail throughout this book.

## *Suggested Readings*

Hille B. Ionic Channels of Excitable Membranes. 3rd Ed. Sunderland, MA: Sinauer Associates, 2001. (*Clearly discusses the basis of cellular excitability and the biology of ion channels.*)

Conley EC, Brammer WJ. The Ion Channel Factsbook, vol. IV: Voltage-Gated Channels. San Diego: Academic Press, 1996.

Nestler EJ, Hyman SE, Malenka RC. Molecular Neuropharmacology: A Foundation for Clinical Neuroscience. New York: McGraw-Hill/Appleton & Lange, 2001.

# 6

# Principles of Nervous System Physiology and Pharmacology

Joshua M. Galanter and Daniel H. Lowenstein

**Introduction**
**Case**
**Neuroanatomy**
  Anatomy of the Peripheral Nervous System
    *Autonomic Nervous System*
    *Peripheral Motor and Sensory Nerves*
  Anatomy of the Central Nervous System
    *Cerebrum*
    *Diencephalon*
    *Cerebellum*
    *Brainstem*
    *Spinal Cord*
  Cellular Organization of the Nervous System
    *Long Tract Neuronal Organization*
    *Local Circuit Neuronal Organization*
    *Single-Source Divergent Neuronal Organization*
**Neurophysiology**
  Neurotransmitters
    *Amino Acid Neurotransmitters*
    *Biogenic Amines*
    *Other Small Molecule Neurotransmitters*
    *Neuropeptides*
  The Blood–Brain Barrier
**Conclusion**
**Suggested Reading**

## INTRODUCTION

The nervous system contains more than ten billon neurons. Most neurons form thousands of synaptic connections, giving the nervous system complexity unlike that seen in any other organ system. Interactions among neuronal circuits mediate functions ranging from primitive reflexes to language, mood, and memory. To perform these functions, the individual neurons that comprise the nervous system must be organized into functional networks, which, in turn, are organized into larger anatomical units.

The previous chapter reviewed the physiology of individual neurons by describing electrical conduction within a neuron and chemical conduction from one neuron to another. This chapter discusses neuronal systems by examining two levels of organization. First, the gross anatomical organization of the nervous system is presented, to place in context the sites of action of pharmacologic agents that act on this system. Second, the major patterns of neuronal connectivity (so-called neuronal tracts) are presented because knowledge of the ways in which neuronal cells are organized to transmit, process, and modulate signals facilitates a deeper understanding of the actions of drugs on these tracts. This chapter also discusses the neurophysiology of specific neurotransmitters and the blood–brain barrier; these functional and metabolic concepts have important pharmacologic consequences for drugs that act on the nervous system.

Although this chapter is designed to focus on concepts relevant to neuropharmacology, it is obviously not intended as a replacement for a neuroscience course. Instead, the goal of this chapter is to provide sufficient anatomical and physiological background to understand the neuropharmacology presented in the rest of the book.

## Case

Martha P is a 66-year-old woman with a 4-year history of worsening Parkinson's disease, which is a neurological disorder resulting from the progressive degeneration of nigrostriatal neurons that use dopamine as a neurotransmitter. The disease causes a resting tremor, rigidity, difficulty initiating movement, and postural instability. While visiting her physician, Ms P registers an unusual complaint:

"It seems like my Sinemet doesn't work as well when I take it with meals." Ms P explains that she has recently started on a new "low-carb" diet that has increased her protein intake at the expense of high-carbohydrate foods. Concerned, Ms P asks, "Could my diet have anything to do with this?" Her physician explains that levodopa, a component of the medicine she takes, helps replace a chemical in her brain that is produced in insufficient quantities because of the loss of certain neurons in her brain. Although many factors could lead to the decreased effectiveness of her medication, he confirms her suspicion that her high-protein diet could be interfering with the medication's ability to reach her brain. Ms P's doctor recommends that she moderate her protein intake, and, if necessary, take a higher dose of Sinemet after a high-protein meal. On her follow-up visit, Ms P is happy to report that her medication is more effective now that she is eating less protein.

## QUESTIONS

1. Where is the nigrostriatal tract located? How does the degeneration of a specific group of neurons result in specific symptoms such as those seen in Parkinson's disease?
2. Why is levodopa used in the treatment of Parkinson's disease, and what is the relationship of this compound to dopamine?
3. Why does protein consumption interfere with the ability of levodopa to be transported into the brain?

## NEUROANATOMY

The nervous system can be divided structurally and functionally into **peripheral** and **central** components. The peripheral nervous system includes all nerves traveling between the central nervous system and somatic and visceral sites. It is divided functionally into the **autonomic** (involuntary) nervous system and the **sensory and somatic** (voluntary) nervous system.

The central nervous system (CNS) includes the cerebrum, diencephalon, cerebellum, brainstem, and spinal cord. The CNS relays and processes signals received from the peripheral nervous system; the processing results in a response that is formulated and relayed back to the periphery. The CNS is responsible for important functions such as perception—including sensory, auditory and visual processing—wakefulness, language, and consciousness.

## ANATOMY OF THE PERIPHERAL NERVOUS SYSTEM

The autonomic nervous system regulates involuntary responses of smooth muscle and glandular tissue. As examples, it controls vascular tone, heart rate and contractility, pupillary constriction, sweating, salivation, piloerection ("goose bumps"), uterine contraction, gastrointestinal motility, and bladder function. The autonomic nervous system is divided into the **sympathetic** nervous system, responsible for the "fight or flight" response, and the **parasympathetic** nervous system, responsible for the "rest and digest" response. The sensory and somatic peripheral nervous system carries sensory signals from the periphery to the CNS and motor signals from the CNS to striated muscle; these signals regulate voluntary movement (Fig. 6-1).

### Autonomic Nervous System

*Autonomic nerve fibers interact with their target organs by a two-neuron pathway.* The first neuron originates in the brainstem or spinal cord, and is termed a **preganglionic neuron.** The preganglionic neuron synapses outside the spinal cord with a postganglionic neuron that innervates the target organ. As discussed below, the anatomical location of these connections differs for neurons of the sympathetic and parasympathetic divisions of the autonomic nervous system.

#### *1. Anatomy of the Sympathetic Nervous System*

The sympathetic nervous system is also known as the **thoracolumbar system,** because its preganglionic fibers arise from the first thoracic segment to the second or third lumbar segment of the spinal cord (Fig. 6-2). Specifically, the preganglionic nerve cell bodies arise from the **intermediolateral columns** in the spinal cord. Preganglionic nerves exit the spinal cord at the ventral roots of each vertebral level and make synaptic connections with postganglionic neurons in sympathetic ganglia. Most sympathetic ganglia are located in the sympathetic chains, which consist of 25 pairs of interconnected ganglia that lie on either side of the vertebral column. The first three sympathetic ganglia, whose postganglionic fibers are carried with the cervical spinal nerves, are termed the **superior cervical ganglion, middle cervical ganglion,** and **inferior cervical ganglion.** The superior cervical ganglion innervates the pupil, salivary glands, and lacrimal glands, as well as blood vessels and sweat glands in the head and face (Fig. 6-2). Postganglionic neurons arising in the middle and inferior cervical ganglia, as well as the thoracic ganglia, innervate the heart and lungs. Fibers arising from the remaining paravertebral ganglia innervate sweat glands, pilomotor muscles, and blood vessels of skeletal muscle and skin throughout the body.

Postganglionic neurons that innervate the gastrointestinal tract down to the sigmoid colon, including the liver and pancreas, originate in ganglia that are located anterior to the aorta, at the origins of the celiac, superior mesenteric and inferior mesenteric blood vessels (Fig. 6-2). Hence, these ganglia, together known as **prevertebral ganglia,** are named the **celiac, superior mesenteric,** and **inferior mesenteric ganglia,** respectively. In contrast to the paravertebral ganglia, the prevertebral ganglia have long preganglionic fibers and short postganglionic fibers.

The **adrenal medulla** is contained within the adrenal glands that lie on the superior surface of the kidneys. The adrenal medulla contains postsynaptic neuroendocrine cells (Fig. 6-2). Unlike sympathetic postganglionic neurons, which synthesize and release norepinephrine, neuroendocrine cells of the adrenal medulla synthesize primarily epinephrine (85%) and release this neurotransmitter into the bloodstream rather than at synapses on a specific target organ (see Chapter 8, Adrenergic Pharmacology).

Many pharmacologic agents modulate sympathetic nervous system activity. As discussed in Chapter 8, the sympathetic nervous system has an organ-specific distribution of

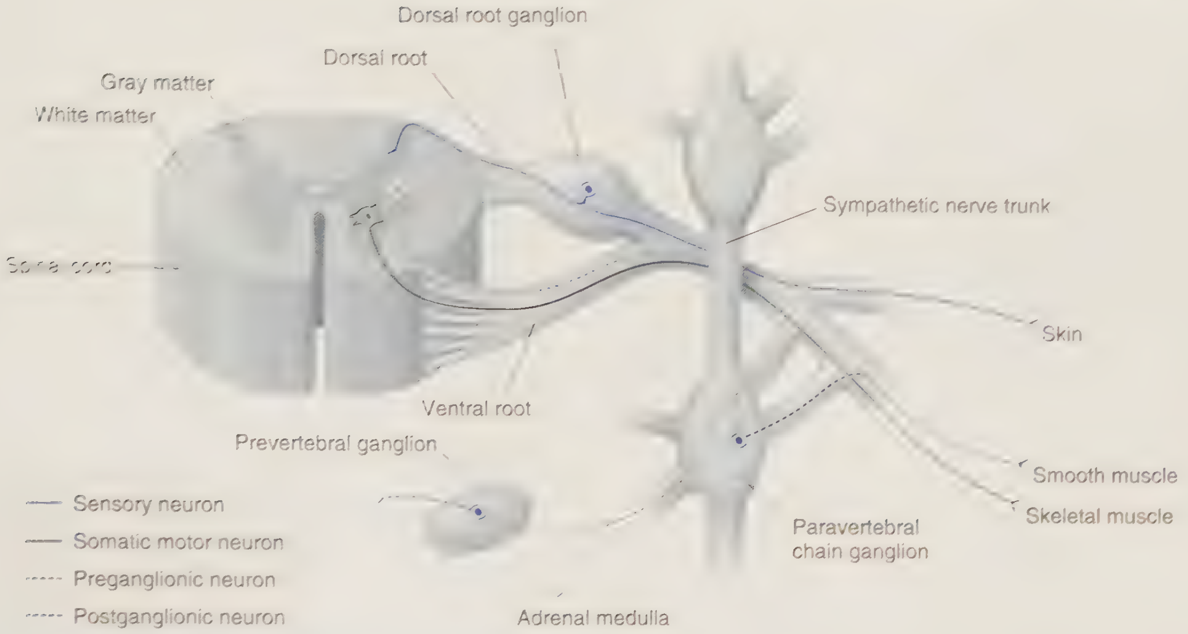


Figure 6-1. Organization of the Peripheral Nervous System. The peripheral nervous system contains sensory, somatic motor, and autonomic components. Sensory neurons (solid blue line) arise principally in the skin or joints, have cell bodies and nuclei in the dorsal root ganglia, and project onto neurons located in the dorsal horn of the spinal cord. Somatic motor neurons (solid black line) arise in the ventral horn of the spinal cord, exit through the ventral roots, and join fibers of sensory neurons to form spinal nerves, which then innervate skeletal muscle. The autonomic component of the peripheral nervous system consists of a two-nerve system; the two nerves are called preganglionic and postganglionic neurons, respectively. Preganglionic neurons (dashed gray line) arise in the ventral horn of the spinal cord and project onto postganglionic neurons in the paravertebral and prevertebral ganglia. The postganglionic neurons (dashed blue line) innervate many organs, including smooth muscle. The adrenal medulla is also innervated by preganglionic neurons of the autonomic nervous system (see Fig. 6-2).

adrenergic receptor types. This organ-specific receptor expression allows drugs to modulate sympathetic activity selectively; for example, certain sympathetic agonists, such as albuterol, can dilate bronchioles selectively, while certain sympathetic antagonists, such as metoprolol, can decrease heart rate and contractility selectively.

### 2. Anatomy of the Parasympathetic Nervous System

Nearly all of the parasympathetic ganglia lie in or near the organs they innervate. The preganglionic fibers of the parasympathetic nervous system arise in the brainstem or in sacral segments of the spinal cord; thus, the parasympathetic system is also called the **craniosacral system** (Fig. 6-2). In some cases, parasympathetic preganglionic neurons can travel almost one meter before synapsing with their postganglionic targets. Preganglionic nerve fibers of cranial nerve (CN) III, the **oculomotor nerve,** arise from a region of the midbrain termed the **Edinger–Westphal nucleus** and innervate the pupil, stimulating it to constrict. The medulla of the brain contains nuclei for parasympathetic nerve fibers in CNs VII, IX, and X. Parasympathetic fibers in the **facial nerve** (CN VII) stimulate salivary secretion by the submaxillary and sublingual glands as well as tear production by the lacrimal gland. Parasympathetic fibers of the ninth cranial nerve, the **glossopharyngeal nerve,** stimulate the parotid gland. The tenth cranial nerve, termed the **vagus nerve,** provides parasympathetic innervation to the major organs in the chest and abdomen, including the heart, tracheobronchial tree, kidneys, and gastrointestinal system down to the proximal colon. Parasympathetic nerves originating in the sacral region of the spinal cord innervate the remainder of the colon, urinary bladder, and genitalia.

Many pharmacologic agents modulate parasympathetic nervous system activity. For example, bethanechol is a parasympathomimetic that promotes GI and urinary tract motility.

Antagonists of parasympathetic activity include atropine, a drug used locally to dilate the pupils or systematically to increase heart rate, and ipratroprium, a drug used to dilate bronchioles. These agents and others are discussed in Chapter 7, Cholinergic Pharmacology.

## Peripheral Motor and Sensory Nerves

Fibers of the somatic nervous system innervate their target striated muscles directly (Fig. 6-1). These neurons originate in the **ventral horns** of the spinal cord, and exit through the **ventral roots.** They join the **dorsal roots,** which carry sensory nerve fibers, to form the **spinal nerves.** Spinal nerves exit the vertebral column through the intervertebral foramina, after which they separate into peripheral nerves. Somatic components of the peripheral nerves innervate muscles directly. Muscles are innervated in a **myotomal distribution.** That is, neurons originating from a particular ventral root level of the spinal cord (e.g., C6) innervate specific muscles (e.g., flexor muscles of the forearm).

Sensory neurons have cell bodies in the **dorsal root ganglia.** The endings of sensory nerves lie in the skin and joints

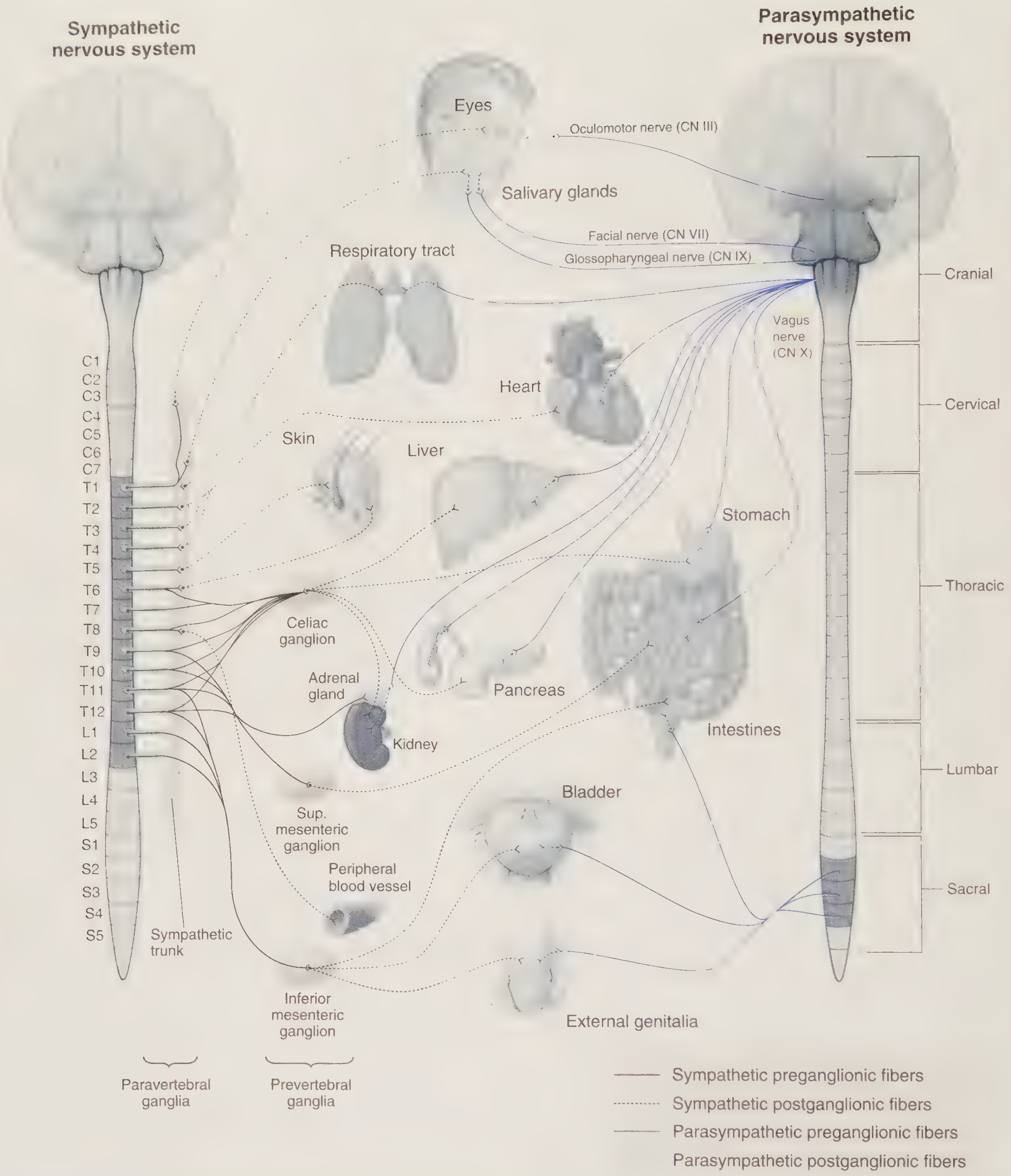

Sympathetic nervous system
Parasympathetic nervous system
Eyes
Oculomotor nerve (CN III)
Salivary glands
Facial nerve (CN VII)
Glossopharyngeal nerve (CN IX)
Respiratory tract
Cranial
Vagus nerve (CN X)
Heart
Cervical
Skin
Liver
Stomach
Thoracic
C1
C2
C3
C4
C5
C6
C7
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
L1
L2
L3
L4
L5
S1
S2
S3
S4
S5
Celiac ganglion
Adrenal gland
Pancreas
Kidney
Intestines
Lumbar
Bladder
Sup. mesenteric ganglion
Peripheral blood vessel
Sacral
Sympathetic trunk
Inferior mesenteric ganglion
External genitalia
Paravertebral ganglia
Prevertebral ganglia
Sympathetic preganglionic fibers
Sympathetic postganglionic fibers
Parasympathetic preganglionic fibers
Parasympathetic postganglionic fibers

**Figure 6-2. Patterns of Sympathetic and Parasympathetic Innervation.** Sympathetic preganglionic neurons arise in the thoracic and lumbar segments of the spinal cord. Sympathetic preganglionic neurons project onto postganglionic neurons in ganglia that lie close to the spinal cord, most notably the paravertebral ganglia, and in the prevertebral ganglia located near the aorta. Parasympathetic ganglia generally lie close to the organs they innervate. Thus, parasympathetic preganglionic neurons, which arise in nuclei in the brainstem and the sacral segments of the spinal cord, are generally long and project onto short postganglionic neurons.

and enter the spinal cord through the **dorsal roots.** Neurons for vibratory and position sense (proprioception) ascend through the **dorsal columns** in the spinal cord and synapse with secondary neurons in the lower medulla. Sensory neurons that carry sensations of pain, temperature, and touch synapse with secondary neurons in the **posterior horn** of the spinal cord. Sensory information is encoded in a **dermatomal distribution.** That is, neurons originating from a particular dorsal root level of the spinal cord (e.g., C6) carry sensory information corresponding to a particular area of the skin (e.g., the lateral aspects of the forearm and hand).

A number of pharmacologic agents modulate the activity of the somatic nervous system. For example, antagonists of neuromuscular junction activity, such as pancuronium, are used to induce paralysis during surgery. In contrast, drugs that increase neuromuscular junction activity, such as edrophonium and neostigmine, are used in the diagnosis and treatment of myasthenia gravis, an autoimmune disease characterized by decreased skeletal muscle stimulation at the neuromuscular junction. These agents and others are discussed in Chapter 7.

## ANATOMY OF THE CENTRAL NERVOUS SYSTEM

The CNS is divided anatomically into seven major divisions; namely, the **cerebral hemispheres, diencephalon, cerebellum, midbrain, pons, medulla,** and **spinal cord** (Fig. 6-3). The midbrain, pons, and medulla are collectively known as the **brainstem,** and together connect the spinal cord with the cerebrum, diencephalon, and cerebellum.

### Cerebrum

The cerebral hemispheres constitute the largest division of the human brain. These structures contain several subdivisions, including the **cerebral cortex,** its underlying **white matter,** and the **basal ganglia** (Fig. 6-4). The cerebral hemispheres are divided into left and right sides that are connected by the **corpus callosum.** The cerebral cortex is responsible for high level functions, including sensory perception, planning and ordering motor functions, cognitive functions, such as abstract reasoning, and language. The cortex is divided anatomically and functionally into the **frontal, temporal, parietal,** and **occipital** lobes (Fig. 6-4A). Subregions of the cortex have specific functions. For example, stimulation of part of the precentral gyrus, which lies in the frontal cortex, induces peripheral motor function (movement), and ablation of this structure inhibits movement. From a pharmacologic perspective, the cerebral cortex is a site of action of many drugs, sometimes as part of their intended mechanism of action and sometimes as a side effect. **Barbiturates** and **benzodiazepines** (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission) are commonly prescribed hypnotics and sedatives that function by activating inhibitory neurons in the cortex. **General anesthetics** (see Chapter 14, General Anesthetic Pharmacology) are also thought to have effects on the cerebral cortex.

The cerebral white matter, which includes the corpus callosum (Fig. 6-4B), transmits signals between the cortex and other areas of the central nervous system and from one area of the cortex to another. The white matter consists primarily of myelinated axons that, as in other areas of the brain, have an associated vascular network of small arteries, veins, and capillaries. It is around these small vessels that inflammatory cells collect in diseases such as multiple sclerosis, and it is the small arterioles that are especially affected by systemic hypertension.

The basal ganglia consist of three deep nuclei of gray matter (Fig. 6-4C), including the **caudate** and **putamen**—together known as the **striatum**—and the **globus pallidus.** In a general sense, these nuclei help initiate and control

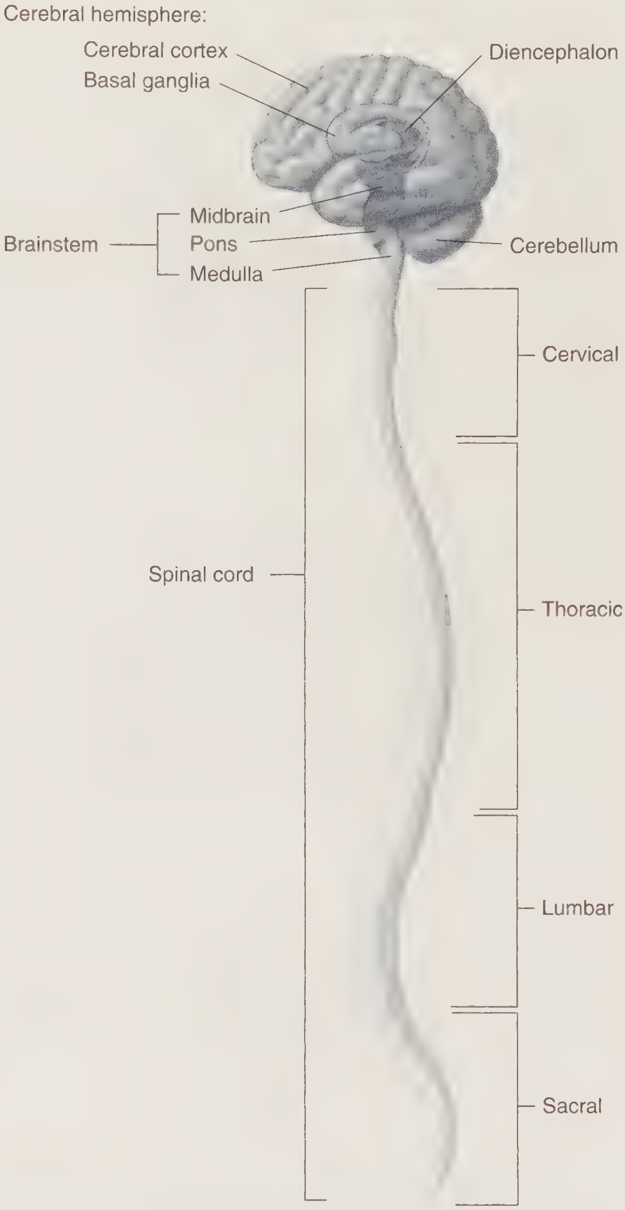


**Figure 6-3. Anatomic Organization of the Central Nervous System.** The central nervous system is divided into seven major regions: the cerebral hemispheres, diencephalon (thalamus), cerebellum, midbrain, pons, medulla, and spinal cord. The cerebral hemispheres include the cerebral cortex, underlying white matter (not shown), and the basal ganglia. The midbrain, pons, and medulla together make up the brainstem. The spinal cord is further divided into cervical, thoracic, lumbar, and sacral segments.

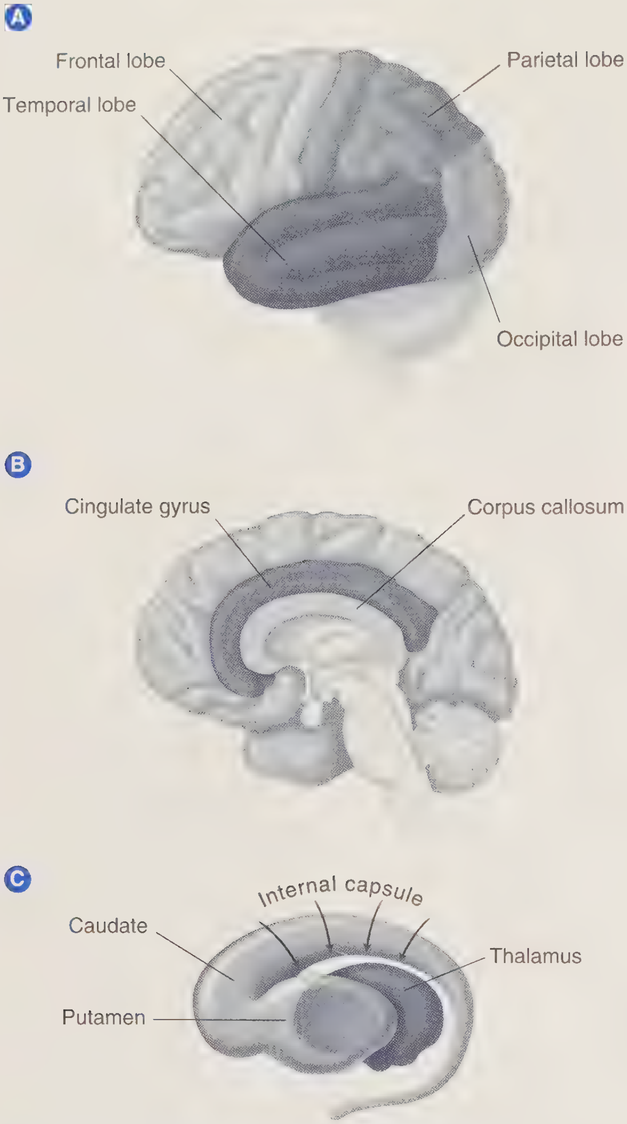


**Figure 6-4. Anatomy of the Cerebral Hemispheres.** **A.** In this lateral view, the cerebral hemispheres are divided into four lobes, including the frontal, parietal, occipital, and temporal lobes, which are structurally and functionally distinct from each other. **B.** A sagittal view of the cerebral hemispheres shows the corpus callosum and the cingulate gyrus. The corpus callosum joins the left and right hemispheres and coordinates their actions. The cingulate gyrus is part of the limbic system; it lies immediately superior to the corpus callosum. **C.** The basal ganglia include the caudate and putamen, which are together known as the striatum, and the globus pallidus (medial to the putamen; not shown). The thalamus lies medial to the basal ganglia. Arrows indicate the trajectory of the internal capsule, a bundle of white matter that carries motor commands from the cortex to the spinal cord.

cortical actions. These actions include not only intended movement, but also behavior and certain rudimentary aspects of cognition. Regions of the basal ganglia responsible for movement ensure that intended actions are carried out, and irrelevant movements are inhibited. As seen in the case of Ms P, Parkinson's disease is caused by degeneration of a dopaminergic pathway that begins in the **substantia nigra** in the midbrain (see below) and terminates in the striatum (hence its name, the **nigrostriatal tract** or pathway). This degeneration prevents the basal ganglia from properly initiating motor activity—resulting in decreased intended movement and an unintended tremor—and causes the decreased affect characteristic of Parkinson's disease. **Levodopa,** a component of Ms P's Sinemet medication, acts on the striatum to ameliorate these clinical manifestations of the disease (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission).

A rim or "*limbus*" around the cortex has "older," more basic functions, and is loosely termed the **limbic system.** This system consists of the **cingulate gyrus** (Fig. 6-4B), the **hippocampal formation** (including the **hippocampus** and surrounding structures), and the **amygdala.** These structures are responsible for emotion, social behavior, autonomic control, the perception of pain, and memory. For example, the memory loss associated with Alzheimer's disease is caused by degeneration of the hippocampal formation. Only a few drugs that affect the limbic system specifically are currently available, although many agents affecting this region of the brain are in development. It should also be noted that many drugs of abuse (see Chapter 16, Pharmacology of Chemical Dependence and Addiction) stimulate the brain reward pathway, which includes the **nucleus accumbens** and its projections to the limbic system.

## Diencephalon

The diencephalon is divided into the **thalamus** and **hypothalamus.** The thalamus, which has several distinct nuclei, is located medially in the brain and inferior to the cerebral cortex. Some thalamic nuclei link sensory pathways from the periphery to the cerebral cortex. Other nuclei act as connections between the basal ganglia and the cortex. The thalamus is not a simple signal relay, but rather it filters and modulates sensory information, in part dictating which signals reach conscious awareness.

The hypothalamus lies ventral to the thalamus. It controls the autonomic nervous system, the pituitary gland, and such essential behaviors as hunger and thermoregulation. Descending pathways from the medial hypothalamus regulate preganglionic autonomic neurons in the medulla and spinal cord. It is believed that the antihypertensive effect of **clonidine** is mediated by its action at receptors on brainstem neurons controlled by the hypothalamus (see Chapter 8). Other neurons originating in the medial hypothalamus secrete hormones either directly into the systemic circulation (e.g., vasopressin from the axon terminals in the posterior pituitary gland) or into a portal system that, in turn, controls hormone secretion by the anterior pituitary gland (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). Finally, the hypothalamus initiates complex behaviors in response to hunger, extremes in temperature, thirst, and time of day.

## Cerebellum

The cerebellum lies inferior to the posterior end of the cerebrum and dorsal to the brainstem. It has three functionally distinct regions: the central **cerebellar vermis,** the lateral **cerebellar hemispheres,** and the small **flocculonodular lobe** (Fig. 6-5). The cerebellum has a relatively well-defined pattern of neural connections, receiving inputs from a wide variety of sources and sending output primarily to the motor areas of the cerebral cortex via the thalamus. The cerebellum coordinates voluntary movements in space and time, maintains balance, controls eye movements, and has roles in motor learning (for example, hand–eye coordination) and certain cognitive functions such as the timing of repetitive events and language. Few drugs are designed primarily to affect the cerebellum. However, several agents, notably alcohol and certain antiepileptic drugs, are toxic to the cerebellum. These agents especially affect the vermis, which controls balance.

## Brainstem

The midbrain, pons, and medulla are collectively known as the **brainstem.** The brainstem connects the spinal cord to the thalamus and cerebral cortex and is arranged with the midbrain superior, the medulla inferior, and the pons bridging the two structures (Fig. 6-3). White matter pathways interconnecting the spinal cord, cerebellum, thalamus, basal ganglia, and cerebral cortex course through this small region of brain. In addition, the brainstem gives rise to most of the **cranial nerves.** Some of these nerves are conduits for sensation from the head and face, including hearing, balance, and taste. The cranial nerves also control the motor output to the skeletal muscles of mastication, facial expression, swallowing, and eye movement. The brainstem also regulates parasympathetic output to the salivary glands and the iris.

The medulla contains several control centers essential for life. These include centers that direct the output of the autonomic nuclei, pacemakers that regulate heart rate and breathing, and centers that control reflex actions such as coughing and vomiting. Several relay structures in the pons also play a role (in conjunction with the midbrain) in regulating vital functions such as respiration. The base of the pons contains white matter tracts connecting the cerebral cortex and the cerebellum. Neurons in the **periaqueductal gray,** especially in the midbrain, send descending projections to the spinal cord that help modulate pain perception (see Chapter 15, Pharmacology of Analgesia).

Clusters of diffusely projecting neurons lie throughout the brainstem, hypothalamus, and the surrounding base of the brain. These nuclei, which include the **locus ceruleus, raphé nucleus,** and several others, comprise the **reticular activating system,** which is responsible for consciousness. Many widely prescribed and illicit drugs influence one or more of these nuclei, which are described in more detail below.

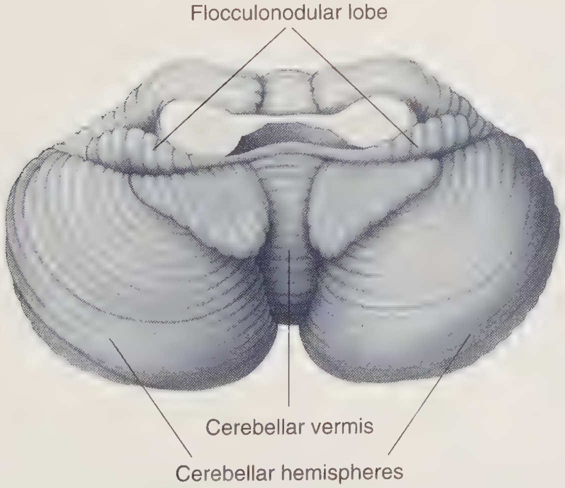


**Figure 6-5. Anatomy of the Cerebellum.** The cerebellum is divided into the cerebellar hemispheres (laterally), the vermis (medially), and the small flocculonodular node. The area immediately above the flocculonodular lobe in this drawing is a cross section of the cerebellar peduncles.

## Spinal Cord

The spinal cord is the most caudal division of the central nervous system. It runs from the base of the brainstem (medulla) at the level of the first cervical vertebra down to the first lumbar vertebra. Like the cerebrum, the spinal cord is organized into white matter tracts and regions of gray matter. The white matter tracts connect the periphery and spinal cord to more rostral divisions of the CNS, while the gray matter forms the nuclear columns that lie in an "H" pattern in the center of the spinal cord (Fig. 6-6).

Neurons in the spinal cord can be defined by their spatial location relative to the gray matter "H." These neurons include sensory neurons, located in the dorsal horns of the H, motor neurons located in the ventral horns of the H, and spinal interneurons. The sensory neurons relay information from the periphery to more rostral divisions of the CNS, while the motor neurons relay commands arising in the central motor areas of the cortex and brainstem to peripheral muscles. Interneurons connect sensory and motor neurons and are responsible for mediating reflexes, such as the deep tendon reflexes, by coordinating the action of opposing mus-

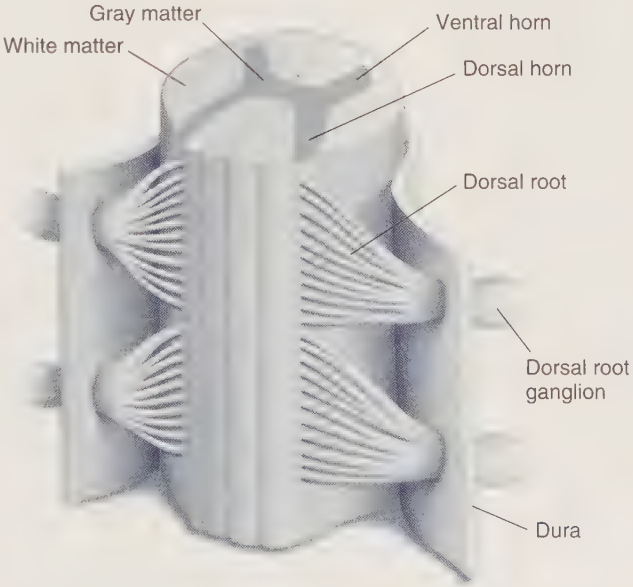


**Figure 6-6. Anatomy of the Spinal Cord.** The spinal cord has an H-shaped wedge of gray matter that includes the dorsal and ventral horns. The dorsal horn is responsible for sensory relays to the brain, and the ventral horn is responsible for motor relays to the skeletal muscle. The white matter carries signals to and from more rostral divisions of the central nervous system.

cle groups. Because the spinal cord carries sensory signals—including sensations of pain—to the central nervous system, it is an important target for analgesic drugs such as the opioids (see Chapter 15).

## CELLULAR ORGANIZATION OF THE NERVOUS SYSTEM

Cellular organization in the autonomic and peripheral nervous system involves a limited number of neurons that make few connections. For example, somatic and sensory information is carried directly between the spinal cord and the periphery. Autonomic nerves are slightly more complex, in that the signal must undergo synaptic transmission between a preganglionic and a postganglionic neuron. In both cases, however, few ancillary neuronal connections are made, and there is little or no modification of information along the way.

In contrast, cellular organization in the central nervous system is far more complex. Information is not simply relayed from one area to another; instead, central neurons receive signals from numerous sources and distribute their own axons widely. Some neurons synapse with hundreds of thousands of other neurons. Moreover, not every synaptic connection is excitatory (i.e., designed to depolarize the postsynaptic neuron). Some connections are inhibitory (i.e., designed to hyperpolarize the postsynaptic neuron). Other neurons projecting onto a target neuron can modulate the relative excitability of the cell, affecting the way that the postsynaptic neuron reacts to other signals. The complexity generated by this variability is needed to carry out the many intricate processes performed by the brain.

Although the CNS possesses immense complexity at the level of neuronal connectivity, three major motifs are used to organize neurons into functional units in the nervous system: the **long tract neuronal systems, local circuits,** and **single-source divergent systems** (Fig. 6-7). The peripheral nervous system is organized exclusively as a long tract system, while the central nervous system uses all three motifs.

### Long Tract Neuronal Organization

*Long tract neuronal organization consists of neural pathways that connect distant areas of the nervous system to one another* (Fig. 6-7A). It is the organization used by the peripheral nervous system and is important for the transmission of signals from one region to another within the central nervous system.

In the peripheral nervous system, signals are transmitted with little modification. Sensory neurons respond to such stimuli as touch, temperature, pressure, vibration, and noxious chemicals, and, if the initial membrane depolarization is strong enough, transmit an action potential directly to the spinal cord. There, sensory neurons synapse directly with somatic motor neurons, forming reflex arcs, and with ascending spinal neurons that transmit the information to higher levels. Motor neurons carry information directly from the spinal cord out through the ventral roots, and project directly on the motor endplates of the muscles they innervate. The long axon tracts of the peripheral neurons are bundled together and travel as peripheral nerves.

As described above, preganglionic neurons of the autonomic nervous system form synaptic connections with postganglionic neurons at ganglia that are located prevertebrally, paravertebrally, or near the innervated visceral organs. One preganglionic neuron typically makes synaptic connections with one to several thousand postganglionic neurons, an organization that is termed "**divergent signaling.**" Although divergent signaling does result in some processing and modification of information, the autonomic nervous system does not generally modify neural signals appreciably.

In contrast to the peripheral pathways, neurons in long tract systems of the central nervous system not only relay but

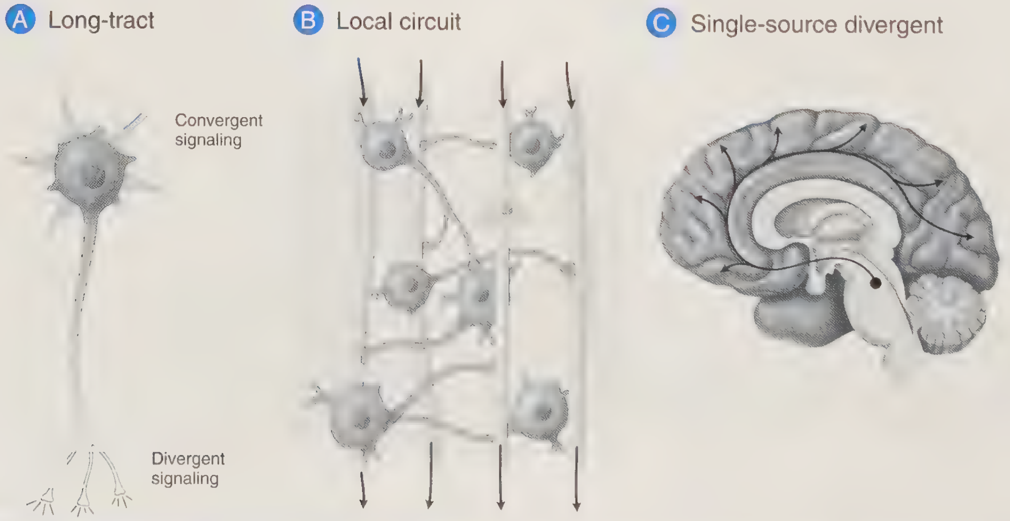


Figure 6-7. Cellular organization of the Central Nervous System. The central nervous system (CNS) has three main organizational motifs. **A.** Long-tract neurons act as relays between the periphery and higher sites in the CNS. Long-tract neurons receive signals from many different neurons (convergence) and synapse with many downstream neurons (divergence). **B.** Local circuit neurons show a complicated structural motif, arranged in layers, which includes both excitatory and inhibitory neurons. These circuits are used to process information. **C.** Single-source divergent neurons typically originate in a nucleus in the brainstem and have axonal terminals that innervate thousands of neurons, usually in the cerebral cortex.

also integrate and modify signals. CNS long tract neurons display divergent signaling like autonomic neurons, but also receive synaptic connections from many upstream neurons (**convergent signaling**). The CNS uses inhibitory neurotransmitters to localize a signal, a strategy that is known as **center-surround signaling.** For example, sensory signaling in the CNS can precisely localize a signal by activating cortical neurons mapping to one area of the body and inhibiting neurons mapping to surrounding areas.

## Local Circuit Neuronal Organization

**Local circuit neurons** *maintain connectivity primarily within the immediate area.* These neurons are generally responsible for modulating signal transmission (Fig. 6-7B). For example, neurons in the cerebral cortex are organized in layers, usually six in number. While information flows into one layer and out of a different layer through long tract connections, links between the layers process the signals and interpret the inputs. Local synaptic connections can be both excitatory and inhibitory, ensuring that only certain patterns of inputs are passed along. For example, information originating in the lateral geniculate neurons enters the primary visual cortex through a long tract connection called the optic tract. In an area of the cortex designed to perceive lines, the outgoing neurons will be excited only if the incoming neurons fire in a particular pattern, in this case designating a line in a particular orientation. The outgoing signal might then serve as the input to another area of the brain that recognizes shapes. If this area receives an appropriate pattern of lines from the appropriate sources, it might recognize a particular object, such as the grid on a tic-tac-toe board.

## Single-Source Divergent Neuronal Organization

Nuclei in the brainstem, hypothalamus, and basal forebrain follow **single-source divergent circuit organization** (Fig. 6-7C), in which *neurons originating in one nucleus innervate many target cells.* Because single-source divergent neuronal organization involves the action of signals on a wide variety of neurons, it is also commonly referred to as a **diffuse system** of organization. Rather than stimulating their targets directly, divergent neurons usually exert a modulatory influence by using neurotransmitters—generally biogenic amines (see below)—that act on G protein-coupled receptors. These receptors alter the resting potential and ion channel conductance of the neuronal membranes in which they are embedded, thereby altering the ease of depolarization of these neurons. Neurons constituting single-source divergent circuits do not generally have myelin sheaths because their modulatory influences vary over the course of minutes or hours rather than fractions of a second. In addition, their axons are highly branched, enabling synaptic connections with a large number of target neurons.

The principal single-source divergent neuronal systems are summarized in Table 6-1. They include pigmented dopaminergic neurons that originate in the **substantia nigra,** widely innervate the striatum, and are responsible for regulating the activity of neurons that control intended actions (Fig. 6-8A). Specifically, neurons in the nigrostriatal pathway excite downstream pathways that initiate movement and inhibit those that suppress movement. The nigrostriatal pathway degenerates in Parkinson's disease, which is why Ms P displayed a paucity of movement. Other dopaminergic neurons medial to the substantia nigra project to the prefrontal cortices and influence thought processes.

Another example of a single-source divergent circuit involves the noradrenergic nucleus in the pons termed the **locus ceruleus** (Fig. 6-8B). Neurons originating in this nucleus widely innervate the cerebral cortex and cerebellum, and maintain vigilance and responsiveness to unexpected stimuli. Thus, drugs such as cocaine, which inhibits the reuptake of catecholamines such as norepinephrine, can activate this system and cause hypervigilance (see Chapter 16).

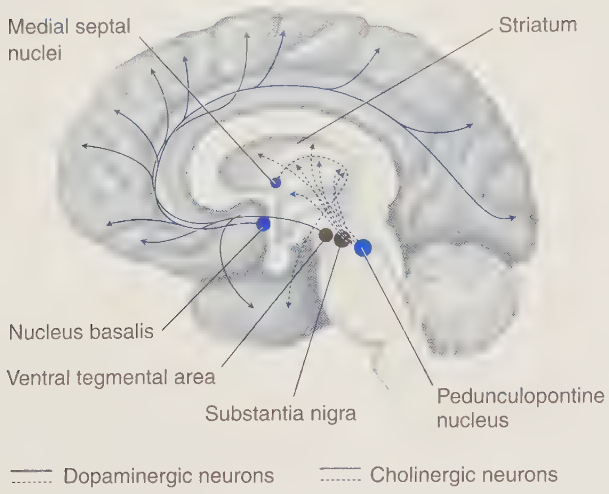
Neurons that originate in the **raphé nucleus** in the caudal brainstem use the neurotransmitter **serotonin** and are responsible for modulating pain signals in the spinal cord and locus ceruleus (Fig. 6-8B). Other neurons originating in the raphé nucleus widely innervate the forebrain, modulating the responsiveness of neurons in the cortex. Serotonergic neurons regulate wakefulness and sleep, and dysfunction of the serotonergic system is hypothesized to be a cause of depression. Because antidepressants block the reuptake of serotonin, it is possible that this class of drugs activates the serotonergic raphé pathway (see Chapter 12, Pharmacology of Affective Disorders).

Three other important nuclei that widely innervate the cortex are the **basal nucleus of Meynert,** the **pedunculo-**

**TABLE 6-1** Single-Source Divergent Neuronal Circuits

| ORIGIN | NEUROTRANSMITTER | FUNCTIONS |
|---|---|---|
| Substantia nigra (midbrain) | Dopamine | Enable intended movement; emotion, thought, memory storage |
| Locus ceruleus (pons) | Norepinephrine | Vigilance; responsiveness to unexpected stimuli |
| Raphé nucleus (medulla, pons, and midbrain) | Serotonin | Perception of pain; responsiveness of cortical neurons; mood (?) |
| Basal nucleus of Meynert | Acetylcholine | Alertness |
| Pedunculopontine nucleus | Acetylcholine | Sleep/wake cycles |
| Tuberomamillary nucleus (hypothalamus) | Histamine | Forebrain arousal |

**A** Dopaminergic and cholinergic pathways



**B** Noradrenergic and serotonergic pathways

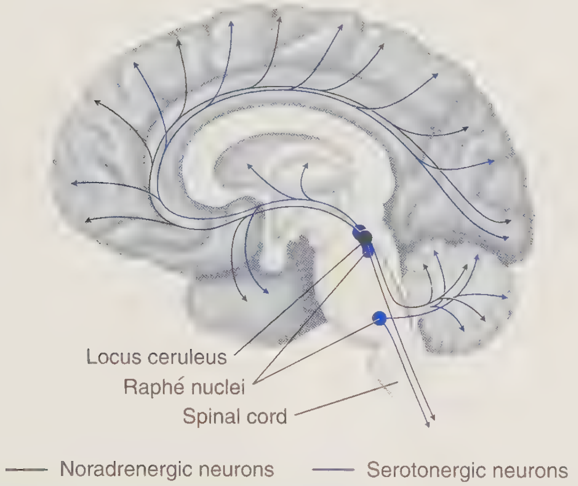


**Figure 6-8. Diffuse Neuronal Systems.** **A.** Dopaminergic neurons (black) arise in the substantia nigra and the ventral tegmental area, and project to the striatum and the cerebral cortex, respectively. These neurons are associated with the initiation of movement and the brain reward pathways. Cholinergic neurons (blue) arise in the nucleus basalis, pedunculopontine nucleus, and medial septal nuclei. These neurons, which project widely throughout the brain, are responsible for maintaining sleep–wake cycles and regulating sensory transmission. **B.** Noradrenergic neurons (black) arise in the locus ceruleus and innervate the entire brain. These neurons are responsible for maintaining alertness. Serotonergic neurons (blue) arise in the raphé nuclei and project to the diencephalon, the basal ganglia and, via the basal forebrain, to the cerebral hemispheres as well as the cerebellum and spinal cord. Serotonergic neurons are believed to have a role in modulating affect and pain.

**pontine nucleus,** and the **tuberomamillary nucleus**. The basal nucleus and the pedunculopontine nucleus use **acetylcholine** as a neurotransmitter (Fig. 6-8A). The former nucleus projects to the cortex and regulates the sleep–wake cycle, while the latter nucleus projects to the intrathalamic neurons that control sensory transmission. Cells in the basal forebrain that receive inputs from the pedunculopontine nucleus degenerate in several diseases, including Alzheimer's disease. The tuberomamillary nucleus uses the neurotransmitter **histamine** (see below), and may help to maintain arousal through its actions on the forebrain. The somnolence induced by the nonspecific histamine blockers used to treat allergies (see Chapter 41, Histamine Pharmacology) may be caused by inhibition of transmission involving tuberomamillary nucleus neurons.

## NEUROPHYSIOLOGY

### NEUROTRANSMITTERS

The peripheral nervous system uses only two neurotransmitters, acetylcholine and norepinephrine (Fig. 6-9). In contrast, the CNS uses not only a wide variety of small molecule neurotransmitters, including acetylcholine and norepinephrine (Table 6-2), but also many **neuroactive peptides.** These peptides may be transmitted concurrently with the small molecule neurotransmitters and have a neuromodulatory role.

The small molecule neurotransmitters can be organized in several broad categories, based on both their structure and function (Fig. 6-10). The first category, the **amino acid** neurotransmitters, include **glutamate, aspartate, $\gamma$-amino butyric acid (GABA),** and **glycine.** The **biogenic amine** neurotransmitters, which are derived from decarboxylated amino acids, include **norepinephrine, dopamine, epinephrine, serotonin,** and **histamine. Acetylcholine,** which is neither an amino acid nor a biogenic amine, is used as a neurotransmitter in both the CNS and the peripheral nervous system. The purines **adenosine** and **adenosine triphosphate** (ATP) are also used in central neurotransmission, although their roles have not been studied in as much detail as those of other neurotransmitters. The lipid soluble gas **nitric oxide** (NO), which has many effects in peripheral tissues, has recently been shown to act as a diffusible neurotransmitter in the CNS.

### Amino Acid Neurotransmitters

The amino acid neurotransmitters are the primary excitatory and inhibitory neurotransmitters in the central nervous system. Two types of amino acid neurotransmitters are used: the acidic amino acids glutamate and aspartate, which are primarily excitatory, and the neutral amino acids GABA and glycine, which are primarily inhibitory. Glutamate, aspartate, and glycine are all alpha-amino acids that are also building blocks for protein synthesis. Glutamate is the primary excitatory neurotransmitter; it acts on both ionotropic (ligand-gated ion channels) and metabotropic (G protein-coupled) receptors (see Chapter 10). Excessive excitation of certain glutamate receptors is one of the mechanisms by which ischemic injury causes neuronal death. For this reason, glutamate receptors are a major target for pharmaceutical research. To date, however, there are few therapeutic agents in clinical use that bind selectively to glutamate receptors. GABA, which is also discussed in Chapter 10, is the primary inhibitory neurotransmitter in the CNS. Several classes of therapeutic drugs, most notably the barbiturates and benzodiazepines, act by binding to GABA receptors and potentiating the effect of endogenous GABA.

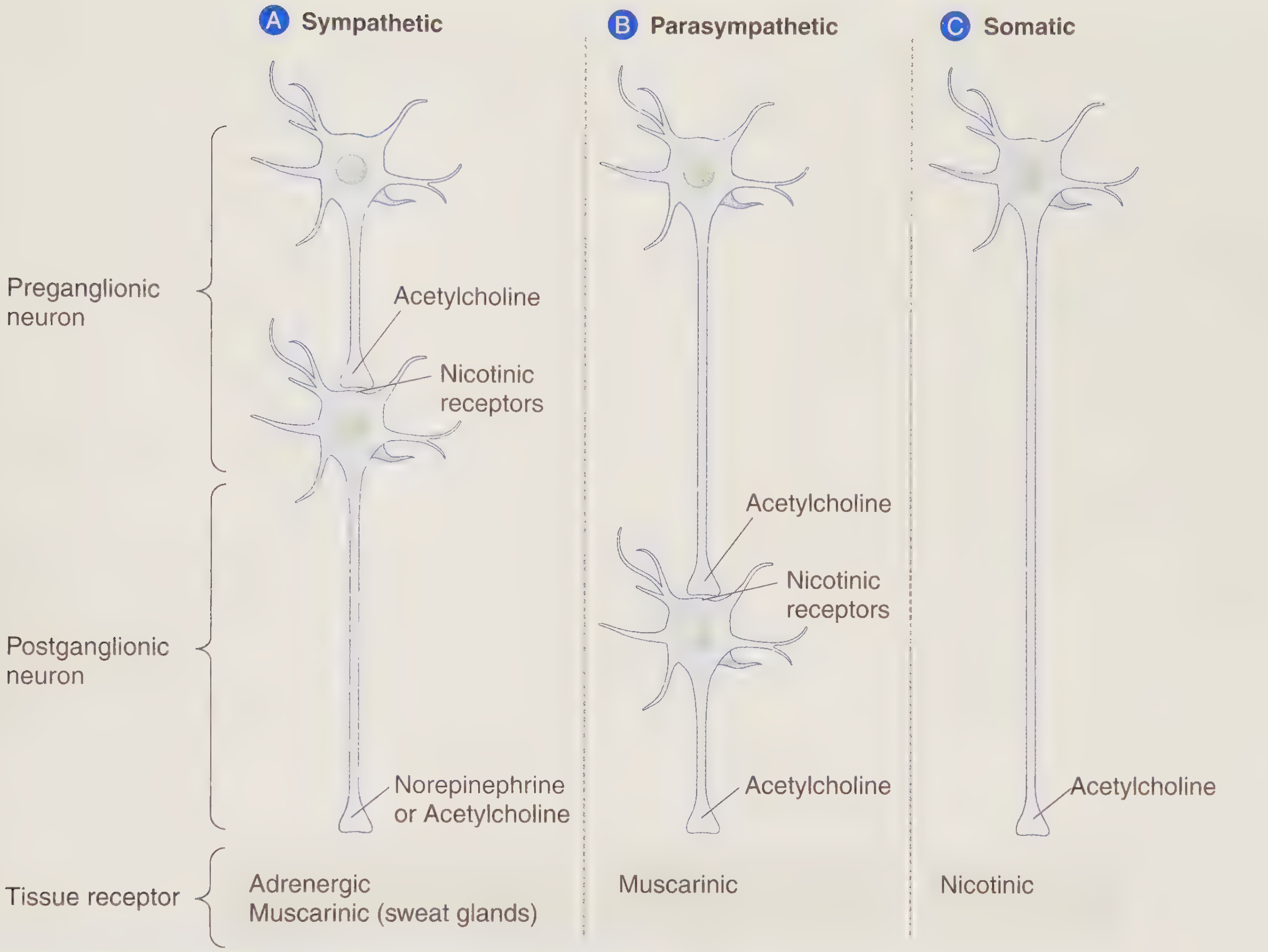


**Figure 6-9. Neurotransmitters Used in the Peripheral Nervous System.** Only two neurotransmitters are required to mediate neurotransmission in the peripheral nervous system. Acetylcholine is released by sympathetic and parasympathetic preganglionic neurons, parasympathetic postganglionic neurons, somatic motor neurons, and sympathetic postganglionic neurons that innervate sweat glands. All other sympathetic postganglionic neurons release norepinephrine. Acetylcholine stimulates nicotinic acetylcholine receptors on sympathetic and parasympathetic postganglionic neurons and at the neuromuscular junction. Acetylcholine stimulates muscarinic acetylcholine receptors on sweat glands and on tissues innervated by parasympathetic postganglionic neurons. Norepinephrine stimulates stimulates α- and β-adrenergic receptors on tissues (except for sweat glands) innervated by sympathetic postganglionic neurons.

## Biogenic Amines

The biogenic amines (along with acetylcholine) are used by the diffuse neuronal systems to modulate such complex central nervous system functions as alertness and consciousness. In the peripheral nervous system, norepinephrine is released by sympathetic postganglionic neurons to mediate the sympathetic response. The adrenal medulla is a neuroendocrine tissue that releases the biogenic amine epinephrine into the circulation in response to stress.

The biogenic amines are all synthesized from amino acid precursors. Based on these precursors, the biogenic amines can be divided into three categories. The **catecholamines** (dopamine, norepinephrine, and epinephrine) are derivatives of tyrosine. The **indoleamine** serotonin is synthesized from tryptophan. **Histamine** is formed from histidine. These three categories are described briefly below.

The catecholamines are all derived from tyrosine in a series of biochemical reactions (Fig. 6-11). First, tyrosine is oxidized to **L-dihydroxyphenylalanine (L-DOPA).** L-DOPA is then decarboxylated to dopamine. In the case of Ms P, L-DOPA (levodopa) is one of the components of the medication used to compensate for the loss of dopaminergic neurons in the substantia nigra. (Dopamine is not effective when administered systematically because it does not cross the blood–brain barrier; see below.) Central dopaminergic receptors have been the target of a wide variety of therapeutics. For example, both dopamine precursors and direct dopamine receptor agonists are used in the treatment of Parkinson's disease, as discussed in Chapter 11. Dopamine receptor antagonists have been used with success in treating the psychotic symptoms of schizophrenia; this topic is also discussed in Chapter 11. Certain drugs of abuse, such as cocaine and the amphetamines, can activate brain reward pathways that depend on dopaminergic neurotransmission, as discussed in Chapter 16.

Dopamine is synthesized from tyrosine and L-DOPA in the cytoplasm, but is then transported into synaptic vesicles. In dopaminergic neurons, dopamine within synaptic vesicles is used directly as the neurotransmitter. In adrenergic and noradrenergic neurons, dopamine is converted to norepinephrine

**TABLE 6-2** Small Molecule Neurotransmitters Used in the Central Nervous System

| NEUROTRANSMITTER | RECEPTOR SUBTYPE | RECEPTOR MOTIF | MECHANISM |
|---|---|---|---|
| GABA | $GABA_A$ | Ionotropic | ↓ cAMP<br>↑ $Cl^-$ conductance |
| | $GABA_B$ | Metabotropic | ↑ $K^+$, $Cl^-$ conductance |
| Glycine | α, β Subunits | Ionotropic | ↑ $Cl^-$ conductance |
| Glutamate, Aspartate | AMPA | Ionotropic | ↑ $Na^+$, $K^+$ conductance |
| | Kainate | Ionotropic | ↑ $Na^+$, $K^+$ conductance |
| | NMDA | Ionotropic | ↑ $Na^+$, $K^+$, $Ca^{2+}$ conductance |
| | mGlu (1–7) | Metabotropic | ↓ cAMP<br>↑ $IP_3$/DAG/$Ca^{2+}$ |
| Dopamine | D1, D5 | Metabotropic | ↑ cAMP |
| | D2, D3, D4 | Metabotropic | ↓ cAMP; ↑ $K^+$, ↓ $Cl^-$ conductance |
| Norepinephrine | $\alpha_1$ | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |
| | $\alpha_2$ | Metabotropic | ↓ cAMP; ↑ $K^+$, ↓ $Cl^-$ conductance |
| | $\beta_1$, $\beta_2$, $\beta_3$ | Metabotropic | ↑ cAMP |
| Serotonin | $5\text{-}HT_1$ | Metabotropic | ↓ cAMP; ↑ $K^+$ conductance |
| | $5\text{-}HT_2$ | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |
| | $5\text{-}HT_3$ | Ionotropic | ↑ $Na^+$, $K^+$ conductance |
| | $5\text{-}HT_{4-7}$ | Metabotropic | ↑ cAMP |
| Histamine | $H_1$ | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |
| | $H_2$ | Metabotropic | ↑ cAMP |
| | $H_3$ | Unknown | Unknown |
| Acetylcholine | Nicotinic | Ionotropic | ↑ $Na^+$, $K^+$, $Ca^{2+}$ conductance |
| | Muscarinic | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$<br>↓ cAMP; ↑ $K^+$ conductance |
| Adenosine | $P_1$ | Metabotropic | ↓ cAMP; ↓ $Ca^{2+}$, ↑ $K^+$ conductance |
| | $P_{2X}$ | Ionotropic | ↑ $Ca^{2+}$, $K^+$, $Na^+$ conductance |
| | $P_{2Y}$ | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |

Neurotransmitters can be organized into several categories including the amino acids, biogenic amines, acetylcholine, adenosine, and nitric oxide. Each neurotransmitter can bind to one or more receptors. Except for the NO receptor, which is intracellular, the other small molecule receptors are all at the cell surface. These cell surface receptors may be ionotropic or metabotropic. The mechanism of action of each receptor is indicated. In addition to the small molecule neurotransmitters, more than 50 neuroactive peptides have been identified. AMPA, kainate, and NMDA receptors are named after agents that selectively activate them. AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid. NMDA, N-methyl-D-aspartate. cAMP, cyclic adenosine-3′,5′-monophosphate. DAG, diacylglycerol. $IP_3$, inositol-1,4,5-trisphosphate.

within the synaptic vesicles by the enzyme dopamine-β-hydroxylase. In a small number of neurons and in the adrenal medulla, norepinephrine is then transported back into the cytoplasm where it is methylated to form epinephrine. Chapter 8 discusses the pharmacology of drugs that target peripheral adrenergic receptors, including both agonists, such as bronchodilators and vasopressors, and antagonists, such as antihypertensives. Several classes of therapeutic agents act on central norepinephrine or epinephrine receptors. **Clonidine** is a partial agonist that acts on presynaptic $\alpha_2$-receptors. Some **antidepressants** increase the synaptic concentration of norepinephrine by blocking its reuptake (**tricyclic antidepressants, TCAs**), while others increase the intracellular pool of norepinephrine available for synaptic release by inhibiting its chemical degradation (**monoamine oxidase inhibitors, MAOIs**).

**5-Hydroxytryptamine** (5-HT, also known as serotonin) is formed from the amino acid tryptophan, which is first oxidized at the 5 position and then decarboxylated. This sequence of reactions is similar to that used in the synthesis of dopamine, although the enzymes that carry out the reactions are different (Fig. 6-12). Several classes of drugs target serotonergic neurotransmission. Tricyclic antidepressants, which block norepinephrine reuptake, also block serotonin reuptake. **Selective serotonin reuptake inhibitors (SSRIs)**, which act more selectively on serotonin reuptake transporters, are also used to treat depression. The role of serotonergic neurons in depression, and the various therapies for depression that target serotonergic neurotransmission, are discussed in more detail in Chapter 12.

Histamine is formed by decarboxylation of the amino acid histidine. Histamine functions as a diffuse neurotransmitter in the central nervous system, although few therapeutics intentionally target central histaminergic neurotransmission. Many antihistamines are designed to act on peripheral histamine $H_1$ receptors; however, where histamine mediates the inflammatory response to allergic stimuli. Several therapeutics that target histamine $H_2$ receptors are used in the treatment of peptic ulcer disease (see Chapter 41). As mentioned above, the action of histamine $H_1$ receptor antagonists on central histamine receptors may produce the side effect of drowsiness.

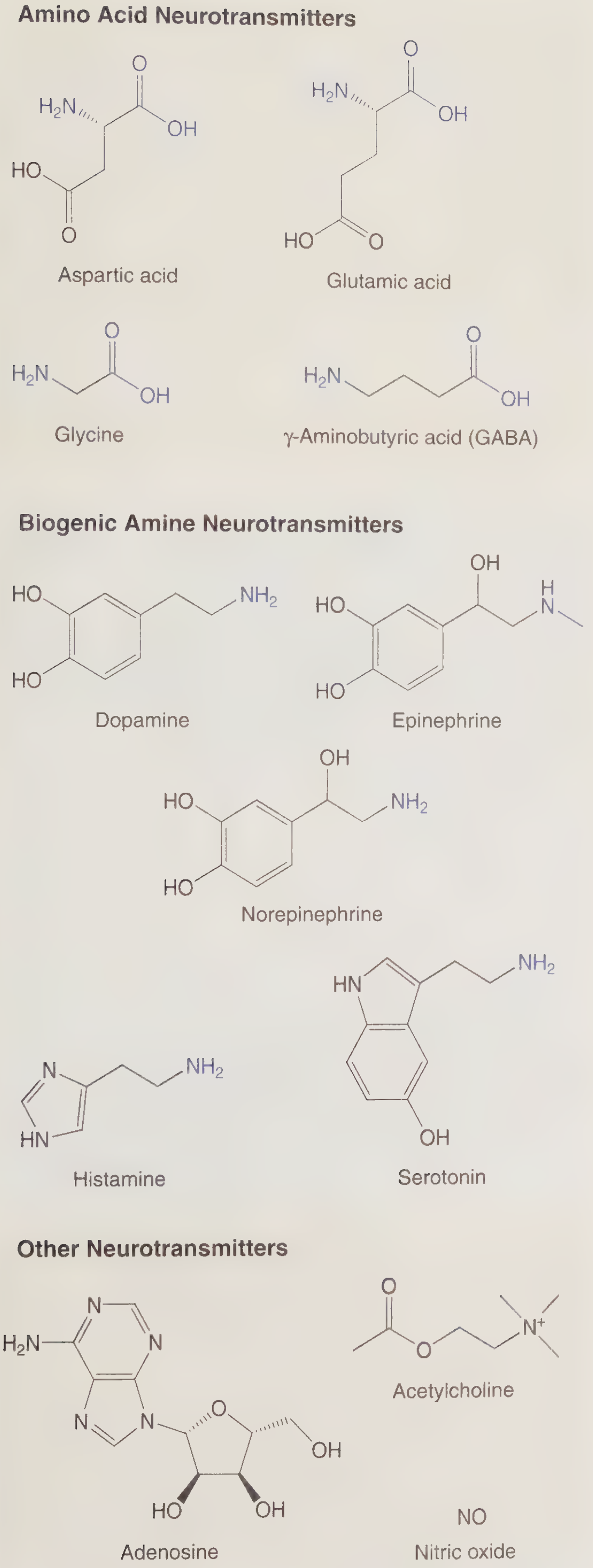


**Figure 6-10. Structures of the Small Molecule Neurotransmitters.** The principal small molecule neurotransmitters can be divided into two broad categories. Amino acids are the primary excitatory (glutamate and aspartate) and inhibitory (glycine and γ-aminobutyric acid) neurotransmitters in the central nervous system (CNS). Their amino and carboxylic acid groups are shown in blue. Biogenic amines are the primary modulatory neurotransmitters in the CNS. The amine moiety is shown in blue. Dopamine, norepinephrine, and epinephrine share a common catechol group, and histamine has an imidazole and serotonin has an indole group. Acetylcholine, a neurotransmitter in diffuse modulatory systems in the CNS, adenosine, and nitric oxide (NO) do not fall into either structural category. The bond order is 2.5 for the nitrogen–oxygen bond in NO, intermediate in strength between a double bond and a triple bond.

## Other Small Molecule Neurotransmitters

Acetylcholine plays a major role in peripheral neurotransmission. At the neuromuscular junction, this molecule is used by somatic motor neurons to depolarize striated muscle. In the autonomic nervous system, acetylcholine is the neurotransmitter used by all preganglionic neurons and by parasympathetic postganglionic neurons. The multiple functions of acetylcholine in the peripheral nervous system have spurred the development of a wide range of medications that target peripheral acetylcholine neurotransmission. These include muscle paralytics, which interfere with neurotransmission at the motor endplate, acetylcholinesterase inhibitors, which increase local acetylcholine concentration by interfering with the metabolic breakdown of the neurotransmitter, and receptor-specific agonists and antagonists.

In the central nervous system, acetylcholine acts as a diffuse system neurotransmitter. Like the biogenic amines, it is thought to regulate sleep and wakefulness. **Donepezil,** a reversible acetylcholinesterase inhibitor that acts on central cholinergic neurons, helps to "brighten" patients with dementia (see Chapter 7). Peripheral anticholinergic agents may cause central cholinergic blockade and, thereby, result in major side effects. For example, the antimuscarinic drug **scopolamine** can cause drowsiness, amnesia, fatigue, and dreamless sleep. In contrast, cholinergic agonists such as **pilocarpine** can induce side effects of cortical arousal and alertness.

The **purinergic** neurotransmitters adenosine and adenosine triphosphate have a role in central neurotransmission. This is most evident in the effects of **caffeine,** which competitively inhibits adenosine receptors and causes a mild stimulant effect. In this case, the adenosine receptors are located on presynaptic noradrenergic neurons and act to inhibit the release of norepinephrine. Inhibition of the activity of these adenosine receptors by caffeine causes the release of norepinephrine to be disinhibited, which causes the characteristic stimulatory effects of the drug.

Nitric oxide, which has generated significant interest as a peripheral vasodilator, acts in the brain as a neurotransmitter. Unlike the other small molecule neurotransmitters, NO diffuses through the neuronal membrane and binds its receptors within the target cell. Many receptors for NO are thought to reside in presynaptic neurons, allowing NO to act as a retrograde messenger. While many therapeutics target the peripheral vasodilatory effects of NO, none, as of yet, target its actions as a central neurotransmitter.

## Neuropeptides

The neuroactive peptides are the last major class of neurotransmitters. Many neuropeptides also have endocrine, au-

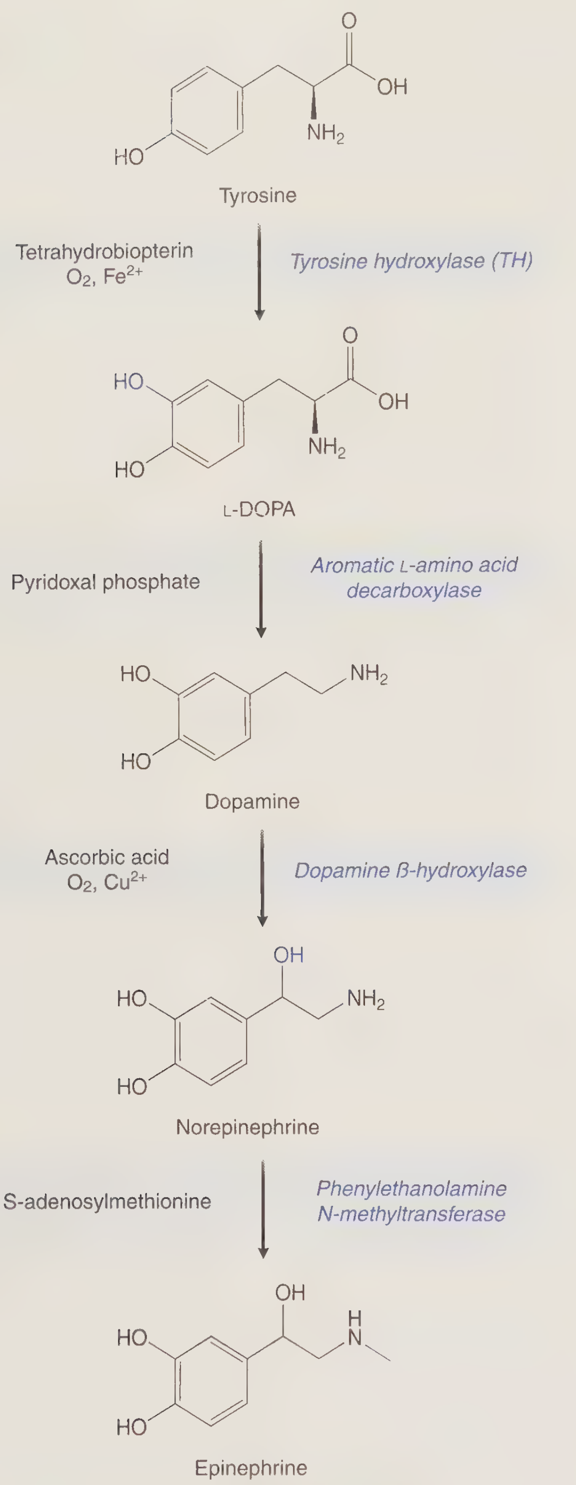

Tyrosine
Tetrahydrobiopterin
$O_2$, $Fe^{2+}$
Tyrosine hydroxylase (TH)
L-DOPA
Pyridoxal phosphate
Aromatic L-amino acid decarboxylase
Dopamine
Ascorbic acid
$O_2$, $Cu^{2+}$
Dopamine β-hydroxylase
Norepinephrine
S-adenosylmethionine
Phenylethanolamine N-methyltransferase
Epinephrine

**Figure 6-11. Synthesis of Catecholamines.** Catecholamines are all synthesized from tyrosine. Sequential enzymatic reactions result in hydroxylation of tyrosine to form L-DOPA, decarboxylation of L-DOPA to form dopamine, hydroxylation of dopamine to form norepinephrine, and methylation of norepinephrine to form epinephrine. Depending on the enzymes (shown in blue lettering) available in a particular type of presynaptic neuron, the reaction sequence may stop at any of the last three steps, so that dopamine, norepinephrine, or epinephrine can be the final product that is synthesized and used as a neurotransmitter.

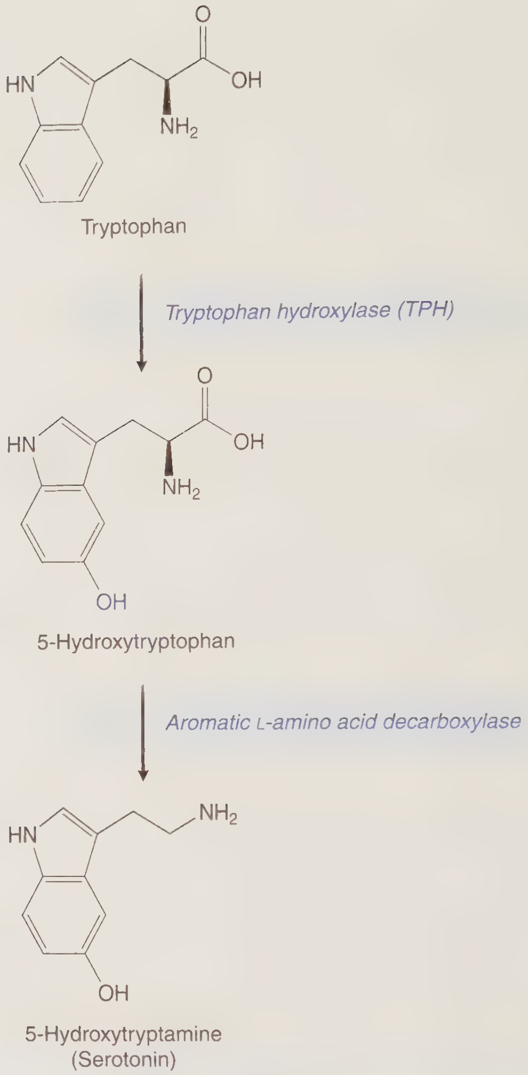

Tryptophan
Tryptophan hydroxylase (TPH)
5-Hydroxytryptophan
Aromatic L-amino acid decarboxylase
5-Hydroxytryptamine
(Serotonin)

**Figure 6-12. Synthesis of 5-Hydroxytryptamine (Serotonin).** Tryptophan is first oxidized by tryptophan hydroxylase (TPH) and then decarboxylated by aromatic L-amino acid decarboxylase to yield serotonin.

tocrine, and paracrine actions. Major examples of neuroactive peptide families are the **opioids,** the **tachykinins,** the **secretins,** the **insulins,** and the **gastrins.** Neuropeptides also include the pituitary hormone release and inhibiting factors, including **corticotropin-releasing hormone (CRH), gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), growth hormone-releasing hormone (GRH)** and **somatostatin.** The opioid peptide family includes the **enkephalins, dynorphins,** and **endorphins.** Opioid receptors, which are distributed widely in areas of the spinal cord and brain that are involved in pain sensation, are the principal pharmacologic targets of such opioid analgesic drugs as morphine (see Chapter 15) and some drugs of abuse such as heroin (see Chapter 16).

## THE BLOOD–BRAIN BARRIER

In the case of Ms P, levodopa (L-DOPA), the immediate precursor of dopamine, was administered rather than the neurotransmitter itself. Unlike L-DOPA, which is able to cross from the blood to the brain tissue where it acts to treat Ms P's Parkinson's disease, dopamine is unable to cross that boundary. The reason for this exclusion is the existence of a selective filter, termed the **blood–brain barrier,** which regulates the transport of many molecules from the blood into the brain (Fig. 6-13). The blood–brain barrier protects the brain tissue both from toxic substances that circulate in the blood and from such neurotransmitters as epinephrine, norepinephrine, glutamate, and dopamine that have systemic effects in body tissues but that would bind receptors in the CNS and cause undesirable side effects if access were permitted.

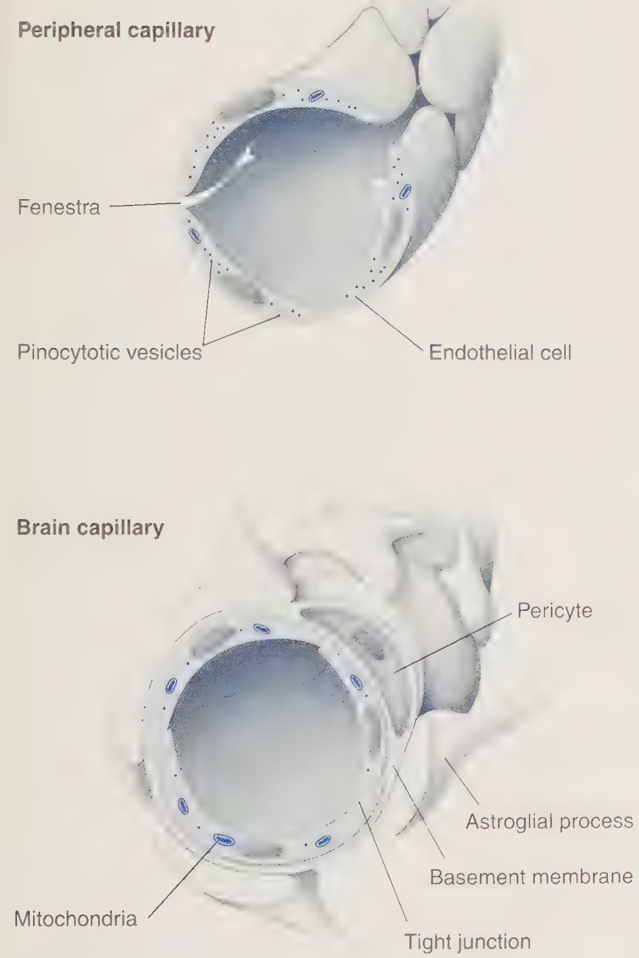


**Figure 6-13. Features of Capillaries in the Central Nervous System Compared to the Peripheral Vasculature.** In the periphery, blood vessel endothelial cells have gaps (termed fenestrae) between them, and intracellular pinocytotic vesicles that facilitate the transcapillary transport of fluid and soluble molecules. In contrast, CNS vessels are sealed by tight junctions between the endothelial cells. The cells have fewer pinocytotic vesicles, and are surrounded by pericytes and astroglial processes. In addition, capillary endothelial cells in the central nervous system have more mitochondria than those in systemic vessels; these mitochondria may reflect the energy requirements necessary for CNS endothelial cells to transport certain molecules into the CNS and transport other molecules out of the CNS.

The structural basis for the blood–brain barrier resides in the unique design of the cerebral microcirculation. In most tissues, there are small gaps, called **fenestrae,** between the endothelial cells that line the microvasculature. These gaps allow water and small molecules to diffuse across the lining without resistance, but filter out large proteins and cells. In the central nervous system, the endothelial cells form tight junctions and, unlike peripheral endothelial cells, do not generally have pinocytotic vesicles that transport fluid from the blood vessel lumen to the extracellular space. In addition, blood vessels in the CNS are covered by cellular processes derived from **astroglia.** These processes play an important role in selectively transporting nutrients from the blood to central neurons.

In the absence of a selective transport mechanism, the blood–brain barrier generally excludes water-soluble substances. In contrast, lipophilic substances, including important lipid-soluble gases, such as oxygen and carbon dioxide, can usually diffuse across the endothelial membranes. The oil–water partition coefficient is a good indicator of the ease with which a small molecule can enter the CNS. Lipophilic substances with high oil–water partition coefficients can generally diffuse across the blood–brain barrier; whereas, hydrophilic substances with low oil–water partition coefficients are typically excluded (Fig. 6-14).

Many important hydrophilic nutrients, such as glucose and a number of amino acids, would not be able to cross the blood–brain barrier without the existence of specific transporters. Glucose, for example, is transported across the barrier by a **hexose transporter** that allows this nutrient to move down its concentration gradient in a process called **facilitated**

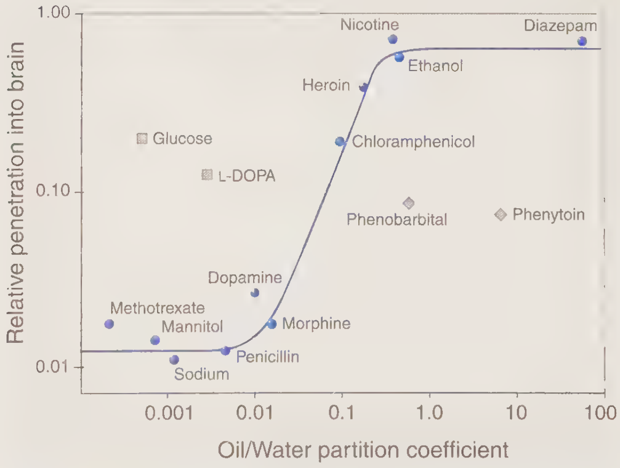


**Figure 6-14. Relative Ability of Compounds to Enter the Brain from the Blood.** In general, there is a correlation between the oil/water partition coefficient of a compound and its ability to enter the brain from the systemic circulation. Specific transporters facilitate the entry into the brain of certain compounds (indicated by squares) such as glucose (glucose transporter) and L-DOPA (aromatic L-amino acid transporter). Transporters also pump certain compounds out of the CNS (indicated by diamonds), such as phenobarbital and phenytoin. The metabolic blood–brain barrier, consisting of a number of drug-metabolizing enzymes, also limits the CNS concentrations of certain drugs.

**diffusion.** Amino acids are transported by three different transporters: one for large neutral amino acids such as valine and phenylalanine; one for smaller neutral amino acids and polar amino acids, such as glycine and glutamate, respectively; and one for alanine, serine, and cysteine. L-DOPA is transported by the large neutral amino acid transporter. For this reason, L-DOPA (levodopa) is administered in lieu of dopamine to patients with Parkinson's disease, and dopamine itself is excluded by the blood–brain barrier. After meals with a high protein content, however, the transporter can become overwhelmed, and its transport of L-DOPA can become ineffective. This explains Ms P's complaint that her medication was less effective after she began a diet high in protein. The blood–brain barrier also contains a number of ion channels, which ensure that ion concentrations in the brain are maintained at homeostatic levels.

Just as certain vital hydrophilic nutrients are allowed access to the brain tissue via particular transporters, many potentially toxic lipophilic compounds can be excluded from the brain by a class of proteins known as **multiple drug resistance (MDR) transporters.** These transporters pump hydrophobic compounds out of the brain and back into the blood vessel lumen. (Note that MDR transporters are present in many cell types, playing an important role in processes such as the resistance of tumor cells to chemotherapeutic agents.) In addition, a **metabolic blood brain–barrier** is maintained by enzymes that metabolize compounds transported into CNS endothelial cells, adding a layer of protection against toxic compounds. One such enzyme, **dopamine decarboxylase,** metabolizes peripheral L-DOPA to dopamine, which is unable to cross the blood–brain barrier. For this reason, Ms P's medication includes a second component, carbidopa, which is an inhibitor of dopamine decarboxylase. Carbidopa ensures that L-DOPA transported from the blood vessel lumen is not metabolized to dopamine peripherally before crossing the blood–brain barrier. Importantly, carbidopa is unable to cross the blood–brain barrier and, therefore, does not interfere with the conversion of L-DOPA to dopamine in the CNS.

## *Conclusion and Future Directions*

This chapter has discussed the anatomical organization of the nervous system and some of the ways in which neurons in the peripheral and central nervous systems are organized. It also describes how neurons are organized to transmit and process electrical and chemical signals, as well as the principal neurotransmitters used by central nervous system neurons. Finally, the chapter explains how drugs are transported to the brain from the bloodstream and how the blood–brain barrier segregates the two compartments.

Although this chapter has introduced some specific drugs as examples, the focus has been on the general principles of anatomy and neurotransmission that are important for understanding the action of all pharmacologic agents that affect the nervous system. The remaining chapters in this section discuss specific neurotransmitter systems and specific agents that act on the peripheral and central nervous systems. Thus, Chapters 7 and 8 describe peripheral cholinergic and adrenergic systems, and Chapter 9 discusses the production of local anesthesia by inhibition of electrical conduction through peripheral sensory neurons. Chapter 10 describes central excitatory and inhibitory neurotransmission. Although few therapeutics currently take advantage of glutaminergic neurotransmission, two major classes of drugs, the benzodiazepines and the barbiturates, affect GABAergic neurotransmission by potentiating the effect of GABA on its receptor. Chapter 11 discusses dopaminergic systems, describing in more detail the concept, introduced in the present chapter, that some of the symptoms of Parkinson's disease can be alleviated by drugs that increase dopaminergic transmission. Chapter 11 also notes that inhibiting dopaminergic transmission can alleviate some of the symptoms of schizophrenia, implying that dopamine may play a role in this disease. Chapter 12 discusses drugs that modify affect, the outward manifestations of mood. These agents include antidepressants, which block reuptake or inhibit metabolism of the biogenic amines norepinephrine and serotonin, as well as the "mood stabilizer" lithium, which is thought to affect a signal transduction pathway. Chapter 13 explores the pharmacology of abnormal electrical neurotransmission. This pharmacology includes the channel blockers, such as phenytoin, which block the propagation of action potentials and, thereby, inhibit many types of seizures. Chapter 14 describes the pharmacology of general anesthetics, agents whose mechanism of action remains controversial. Chapter 15 discusses the pharmacology of analgesia, including opioid receptor agonists and nonopioid analgesics. Finally, Chapter 16 focuses on the mechanisms of drug dependence and addiction, the major drugs of abuse, and treatments for addiction.

## *Suggested Reading*

Kandel ER, Schwartz JH, Jessel TM, eds. Principles of Neural Science. 4th Ed. New York: McGraw-Hill, 2000. [*Considered the premier textbook on neurobiology. Describes both neuroanatomy and neurophysiology in significant depth. Of particular note to readers of this book are Parts II (Cell and Molecular Biology of the Neuron), III (Elementary Interactions Between Neurons: Synaptic Transmission), IV (The Neural Basis of Cognition) and Appendix B (Ventricular Organization of Cerebrospinal Fluid: Blood–Brain Barrier, Brain Edema, and Hydrocephalus).*]

# IIb

# Principles of Autonomic and Peripheral Nervous System Pharmacology

# 7

# Cholinergic Pharmacology

Michael S. Chang and Gary R. Strichartz

- **Introduction**
- **Case**
- **Biochemistry and Physiology of Cholinergic Neurotransmission**
  - Synthesis of Acetylcholine
  - Storage and Release of Acetylcholine
  - Cholinergic Receptors
    - *Muscarinic Transmission*
    - *Nicotinic Transmission*
  - Degradation of Acetylcholine
  - Physiologic Effects of Cholinergic Transmission
    - *Neuromuscular Junction*
    - *Autonomic Effects*
    - *CNS Effects*
- **Pharmacologic Classes and Agents**
  - Inhibitors of Acetylcholine Synthesis, Storage, and Release
  - Acetylcholinesterase Inhibitors
    - *Structural Classes*
    - *Clinical Applications*
  - Receptor Agonists
    - *Muscarinic Receptor Agonists*
    - *Nicotinic Receptor Agonists*
  - Receptor Antagonists
    - *Muscarinic Receptor Antagonists*
    - *Nicotinic Receptor Antagonists*
- **Conclusion and Future Directions**
- **Suggested Readings**

## INTRODUCTION

Cholinergic pharmacology is centered on the properties of the neurotransmitter **acetylcholine (ACh)** and the pathways through which ACh exerts its effects on the body. The functions of cholinergic pathways are both complex and diverse, but generally involve the neuromuscular junction, the autonomic nervous system, and the central nervous system. Although acetylcholine has many important physiologic actions, the current therapeutic uses for cholinergic and anticholinergic drugs are limited. This is because of the ubiquitous and complicated nature of cholinergic pathways, and thus, the inherent difficulty in effecting a specific pharmacologic intervention. Other relevant chapters discussing applications of cholinergic pharmacology are Chapter 15, Pharmacology of Analgesia; Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease; and Chapter 45, Integrative Inflammation Pharmacology: Asthma.

## Case

The year is 1744. Virginian settlers capture Chief Opechancanough, warrior chief of the Powhatans and uncle to Pocahontas. Opechancanough is considered a master tactician and has a reputation as a brutal warrior. One colonial correspondence portrays a different picture of the captured chief, however: "The excessive fatigues he encountered wrecked his constitution; his flesh became macerated; his sinews lost their tone and elasticity and his eyelids were so heavy that he could not see unless they were lifted up by his attendants . . . he was unable to walk; but his spirit, rising above the ruins of his body, directed [his followers] from the litter on which he was carried by his Indians." While Opechancanough is confined to a prison in Jamestown, it is discovered that, after a period of inactivity, he is able to raise himself from the ground to a standing position.

It is thought that the story of Opechancanough provides the earliest recorded description of myasthenia gravis, a neuromuscular disease resulting from the autoimmune production of antibodies directed against cholinergic receptors at the neuromuscular junction. In 1934, almost two centuries later, the English physician Mary Broadfoot Walker encounters several patients with similar symptoms. Rationalizing that the patients have symptoms similar to those of tubocurare poisoning, Dr. Walker administers physostigmine (an inhibitor of acetylcholinesterase) to her immobile patients. The results are startling—within minutes, her patients are able to rise and walk across the room. Dr. Walker has discovered the first truly effective medication for myasthenia gravis. Despite the significance of her accomplishment, it is largely ridiculed by the scientific community because the treatment improves the symptoms of myasthenia gravis too rapidly and effectively to be believable. It is not until many years later that the scientific community comes to accept her findings, and Dr. Walker never fully gains the respect of her peers.

## QUESTIONS

1. Why do tubocurare poisoning and myasthenia gravis produce similar symptoms?
2. What is the therapeutic use of tubocurare, if any?
3. How does physostigmine improve the symptoms of myasthenia gravis?
4. Why is it risky to administer physostigmine to all patients presenting with muscle weakness?
5. What are the other therapeutic uses of physostigmine?

## BIOCHEMISTRY AND PHYSIOLOGY OF CHOLINERGIC NEUROTRANSMISSION

Acetylcholine synthesis, storage, and release follow a similar set of steps in all cholinergic neurons. The specific effects of ACh at a particular cholinergic synapse are largely determined by the ACh receptor type at that synapse. Cholinergic receptors are divided into two broad classes. **Muscarinic cholinergic receptors** (mAChR) are G protein-linked and found at the terminal synapse of all parasympathetic postganglionic fibers and a few sympathetic postganglionic fibers, at autonomic ganglia, and in the CNS. **Nicotinic cholinergic receptors** (nAChR) are ligand-gated ion channels that are concentrated postsynaptically at many excitatory synapses. **Acetylcholinesterase,** the enzyme responsible for acetylcholine degradation, is also an important pharmacologic target. In this section, a description of the biochemistry of each of these pharmacologic targets is followed by a discussion of the physiologic effects of acetylcholine at the neuromuscular junction, in the autonomic nervous system, and in the central nervous system.

### SYNTHESIS OF ACETYLCHOLINE

Acetylcholine is synthesized in a single step from acetyl coenzyme A and choline by the enzyme choline acetyltransferase (ChAT):

$$\text{Acetyl Coenzyme A} + \text{Choline} \xrightarrow{\text{ChAT}} \text{Acetylcholine} + \text{Coenzyme A} + H_2O \qquad \text{Equation 7-1}$$

Acetyl coenzyme A for the reaction derives mainly from glycolysis and is ultimately produced by the enzyme pyruvate dehydrogenase. Although the synthesis of acetyl CoA occurs at the inner membrane of mitochondria, choline acetyltransferase is found in the cytoplasm. An interesting question that still has no definite answer is how acetyl CoA reaches choline acetyltransferase to participate in acetylcholine synthesis. It is theorized that citrate serves as the carrier for acetyl CoA from the mitochondrion to the cytoplasm, where the citrate is freed by citrate lyase.

Although some choline may be synthesized in neurons de novo, the quantities produced are insignificant. Instead, choline used for the synthesis of acetylcholine in the CNS arises from one of three other sources. Approximately 35% to 50% of the choline generated by acetylcholinesterase in the synaptic cleft is transported back into the axon terminal, where it comprises about half of the choline used in ACh synthesis. Plasma-based stores of choline may also be transported to the brain as the lipid phosphatidylcholine, which is then broken down to free choline in response to ACh release. (The incorporation of choline into phosphatidylcholine is essential, because choline itself cannot cross the blood–brain barrier.) Finally, choline is also stored in phospholipids as phosphorylcholine, where it can be used when needed.

*The rate-limiting step of ACh synthesis is mediated not by choline acetyltransferase, but rather by the uptake of choline into the neuron.* There are two processes by which choline transport occurs. The first is low-affinity ($K_m = 10$–$100\ \mu M$) facilitated diffusion. This transport system is not saturable and is found in cells that synthesize choline-containing phospholipids, such as the corneal epithelium. Far more important is a sodium-dependent, high-affinity transport system ($K_m = 1$–$5\ \mu M$) that is specific for cholinergic nerve terminals. Because the high-affinity transporter is easily saturated (at concentrations of choline $> 10\ \mu M$), it provides an upper limit on the supply of choline for ACh synthesis. As the rate-limiting step, this transporter is a target for several anticholinergic drugs (e.g., **hemicholinium-3,** see Fig. 7-1).

### STORAGE AND RELEASE OF ACETYLCHOLINE

After its synthesis in the cytoplasm, ACh is transported into synaptic vesicles for storage. An ATPase that pumps protons into the vesicle provides the energy necessary for this process. Transport of protons out of the vesicle (i.e., down the $H^+$ concentration gradient) is coupled to uptake of ACh into the vesicle (i.e., against the ACh concentration gradient) via an ACh-$H^+$ antiport channel. This antiporter is another target for some anticholinergic drugs, such as **vesamicol,** and its inhibition results in a deficit of ACh storage and subsequent release (Fig. 7-1). Along with ACh, cholinergic vesicles contain ATP and heparan sulfate proteoglycans, both of which serve as counter-ions for ACh. By neutralizing the positive charge of ACh, these molecules disperse electrostatic forces that would otherwise prevent dense packing of ACh within the vesicle. (Released ATP also acts as a neurotransmitter, through purinergic receptors, to inhibit the release of ACh and norepinephrine from autonomic nerve endings.)

Release of ACh into the synaptic cleft occurs through fusion of the synaptic vesicle with the plasma membrane. The process is dependent on axon terminal depolarization and the opening of voltage-dependent calcium channels. The increase in intracellular $Ca^{2+}$ facilitates the binding of syntaxin and three SNARE (soluble N-ethylmaleimide-sensitive factor [NSF]-attachment protein receptor) proteins, which together mediate vesicle–membrane attachment and fusion. The result is that the contents of the vesicle are released into the synaptic cleft. (See Chapter 5, Principles of Cellular Excitability and Electrochemical Transmission, for additional details on electrochemical transmission.)

It is now known that there are two stores of ACh that play distinct roles during the process of ACh release. One store, known as the "depot" pool, consists of vesicles positioned near the plasma membrane of the axon terminal. Axonal depolarization causes these vesicles to release ACh. The "reserve" pool serves to refill the depot pool as it is being used. An adequate rate of reserve pool mobilization is required to sustain ACh release for an extended period of time. Of the two stores, the depot pool is replenished first by vesicles loaded with newly synthesized ACh; this process displaces some of the older depot pool vesicles into the reserve pool.

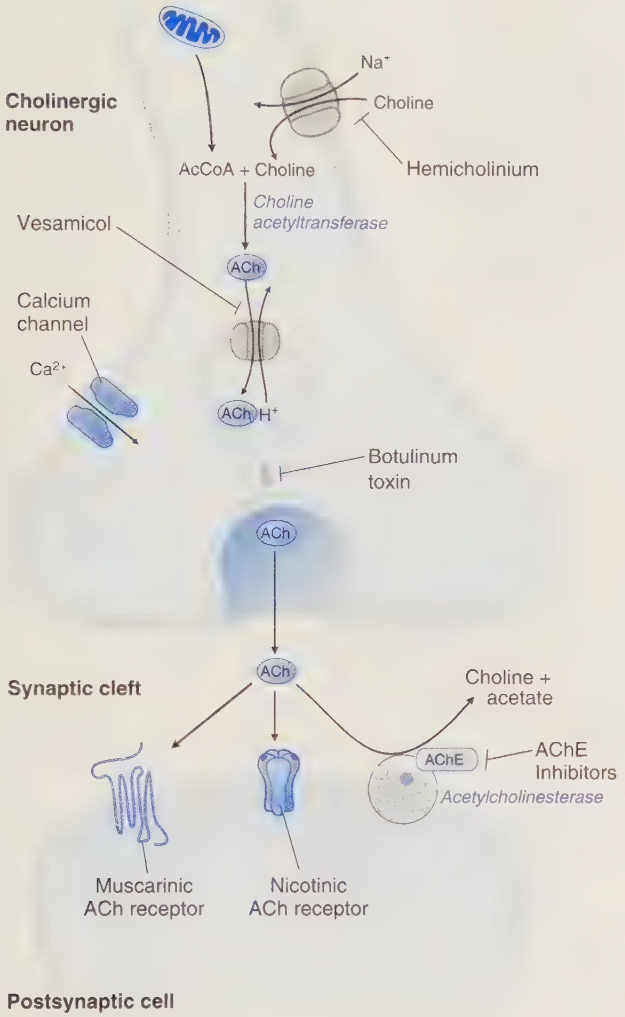


**Figure 7-1. Acetylcholine Synthesis, Storage, Release, and Degradation Pathways, and Pharmacologic Agents that Act on These Pathways.** Choline is transported into the presynaptic cholinergic nerve terminal by a high-affinity $Na^+$-choline cotransporter. This transporter is inhibited by hemicholinium. The cytosolic enzyme choline acetyltransferase then catalyzes the formation of acetylcholine (ACh) from acetyl coenzyme A (AcCoA) and choline. Newly synthesized ACh is packaged (along with ATP and proteoglycans) into vesicles for storage. Transport of ACh into the vesicle is mediated by a $H^+$-ACh antiporter, which is inhibited by vesamicol. The ACh-containing vesicles fuse with the plasma membrane when intracellular calcium levels rise in response to a presynaptic action potential, releasing the neurotransmitter into the synaptic cleft. Botulinum toxin prevents this fusion process, thereby blocking ACh release. Acetylcholine diffuses in the synaptic cleft and binds to postsynaptic and presynaptic receptors. Acetylcholine receptors are divided into nicotinic and muscarinic receptors. Nicotinic receptors are ligand-gated ion channels, while muscarinic receptors are G protein-coupled receptors that alter cell signaling pathways. Acetylcholine in the synaptic cleft is degraded by membrane-bound acetylcholinesterase (AChE) into choline and acetate. Numerous inhibitors of AChE exist; most clinically relevant AChE antagonists are competitive inhibitors of this enzyme.

## CHOLINERGIC RECEPTORS

After ACh has been released into the synaptic cleft, it binds to one of two different classes of receptors, usually on the membrane surface of the postsynaptic cell. **Muscarinic receptors (mAChR)** are seven transmembrane domain G protein-coupled receptors, and **nicotinic receptors (nAChR)** are ligand-gated ion channels.

### Muscarinic Transmission

Muscarinic cholinergic transmission occurs mainly at autonomic ganglia, at end organs innervated by the parasympathetic component of the autonomic nervous system, and in the CNS. *Although muscarinic receptors are sensitive to the same neurotransmitter as nicotinic receptors, these two classes of cholinergic receptors share little structural similarity.* Muscarinic receptors belong to the same family as a number of other cell surface receptors (such as the adrenergic receptors) that transduce signals across the cell membrane and interact with GTP-binding proteins. Because all the effects of muscarinic receptor activation occur through the actions of these G proteins, there is a significant latency of at least 100–250 ms associated with muscarinic responses. (In contrast, nicotinic channels have latencies on the order of 5 ms.)

G protein activation by agonist binding to muscarinic receptors has several effects on the cell. These include inhibition of adenylyl cyclase (via $G_i$) and stimulation of phospholipase C, both mediated by an $\alpha$ subunit of the G protein. (See Chapter 1, Drug–Receptor Interactions, for a discussion of these signaling mechanisms.) Muscarinic activation also influences ion channels. The predominant effect of mAChR stimulation is to increase the opening of specific potassium channels (G protein-modified inwardly rectifying $K^+$ channels, or GIRKs), thereby hyperpolarizing the cell. This effect is mediated through the $\beta\gamma$ subunit of a G protein ($G_o$), which binds to the channel and enhances its probability of being open.

Five distinct cDNAs for human muscarinic receptors have been isolated, and all five receptors, denoted $M_1$–$M_5$, have been detected in vivo. These receptor types form two functionally distinct groups. $M_1$, $M_3$, and $M_5$ are associated with $G_{q/11}$ and, hence, are responsible for the stimulation of phospholipase C. $M_2$ and $M_4$, on the other hand, are coupled to G proteins responsible for adenylyl cyclase inhibition and $K^+$ channel activation. The receptors of each functional group can be distinguished based on their responses to pharmacologic antagonists (Table 7-1). Generally, $M_1$ is associated with cortical neurons and autonomic ganglia, $M_2$ with cardiac muscle, and $M_3$ with smooth muscle and glandular tissue. Because stimulation of $M_1$, $M_3$, and $M_5$ receptors facilitates excitation of the

**TABLE 7-1** Characteristics of Cholinergic Receptor Subtypes

| RECEPTOR | TYPICAL LOCATIONS | RESPONSES | MECHANISM | PROTOTYPE AGONIST | PROTOTYPE ANTAGONIST |
|---|---|---|---|---|---|
| Muscarinic $M_1$ | Autonomic ganglia | Late excitatory postsynaptic potential (EPSP) | $G_{q/11} \rightarrow$ PLC $\rightarrow$ $IP_3$ + DAG $\rightarrow$ $Ca^{2+}$ + PKC | Oxotremorine | Pirenzepine |
| | CNS | Complex: at least arousal, attention, analgesia | | | |
| Muscarinic $M_2$ | Heart: SA node | Slowed spontaneous depolarization; hyperpolarization | βγ of G-protein → inhibit AC and ↑ $K^+$ channel opening | | AF-DX 117 |
| | Heart: AV node | ↓ conduction velocity | | | |
| | Heart: atrium | ↓ refractory period; ↓ contractile force | | | |
| | Heart: ventricle | Slight ↓ in contractility | | | |
| Muscarinic $M_3$ | Smooth muscle | Contraction | As $M_1$ | | Hexahydrosiladifenidol |
| Muscarinic $M_4$ | CNS | | As $M_2$ | | Himbacine |
| Muscarinic $M_5$ | CNS | | As $M_1$ | | |
| Nicotinic $N_M$ | Skeletal muscle neuromuscular end-plates (NMJ) | End-plate depolarization, skeletal muscle contraction | Opening of $Na^+/K^+$ channels | Phenyltrimethylammonium | Tubocurare |
| Nicotinic $N_N$ | Autonomic ganglia | Depolarization and firing of postganglionic neuron | Opening of $Na^+/K^+$ channels | Dimethylphenylpiperazinium | Trimethaphan |
| | Adrenal medulla | Secretion of catecholamines | | | |
| | CNS | Complex: at least arousal, attention, analgesia | | | |

Cholinergic receptors are divided into nicotinic and muscarinic receptors. All nicotinic receptors are ligand-gated cation-selective channels, while muscarinic receptors are G protein-linked transmembrane receptors. Specific pharmacologic agonists and antagonists exist for most subclasses, although the majority of these agents are currently used only for experimental purposes.

cell, while stimulation of $M_2$ and $M_4$ receptors suppresses cellular excitability, there is a predictable correlation between the receptor subtype and the effect of ACh on the cell. The various muscarinic receptor subtypes account for much of the diversity in cellular responses to mAChR agonists.

## Nicotinic Transmission

Nicotinic cholinergic transmission results from the binding of ACh to the nAChR (Fig. 7-2). This phenomenon is known as direct ligand-gated conductance. The simultaneous binding of two ACh molecules to the nAChR elicits a conformational change in the receptor that, in turn, results in the formation of a monovalent cation-selective pore through the cell membrane.

Open channels of the activated nAChR are equally selective to $K^+$ and $Na^+$ ions. Therefore, when open, these channels produce a net inward current that depolarizes the cell. Stimulation of multiple nAChRs may result in the generation of action potentials and the opening of voltage-dependent calcium channels, and produce a number of other effects that accompany cell depolarization.

Because ACh dissociates rapidly from active-state receptor molecules and acetylcholinesterase rapidly degrades free (unbound) ACh in the synaptic cleft (see below), the depolarization mediated by nAChRs is brief (<10 ms). Although the simultaneous binding of two ACh molecules is required for channel opening, it is not necessary for both molecules to dissociate for the channel to open again; binding of a second ACh molecule before departure of the first may, once again, result in channel opening. The kinetics of nAChR binding and channel opening are detailed in Figure 7-3.

Structurally, the nicotinic acetylcholine receptor is comprised of five subunits, each of which has a mass of approximately 40 kilodaltons (Fig. 7-2A). Several types of subunits have been documented in the nAChR, designated α, β, γ, δ, and ε. All of these subunits share 35–50% homology with one another. Each receptor is composed of two α subunits, one β and one δ subunit, and either one γ or one ε subunit. (The $\alpha_2\beta\varepsilon\delta$ form dominates at the neuromuscular junction in mature skele-

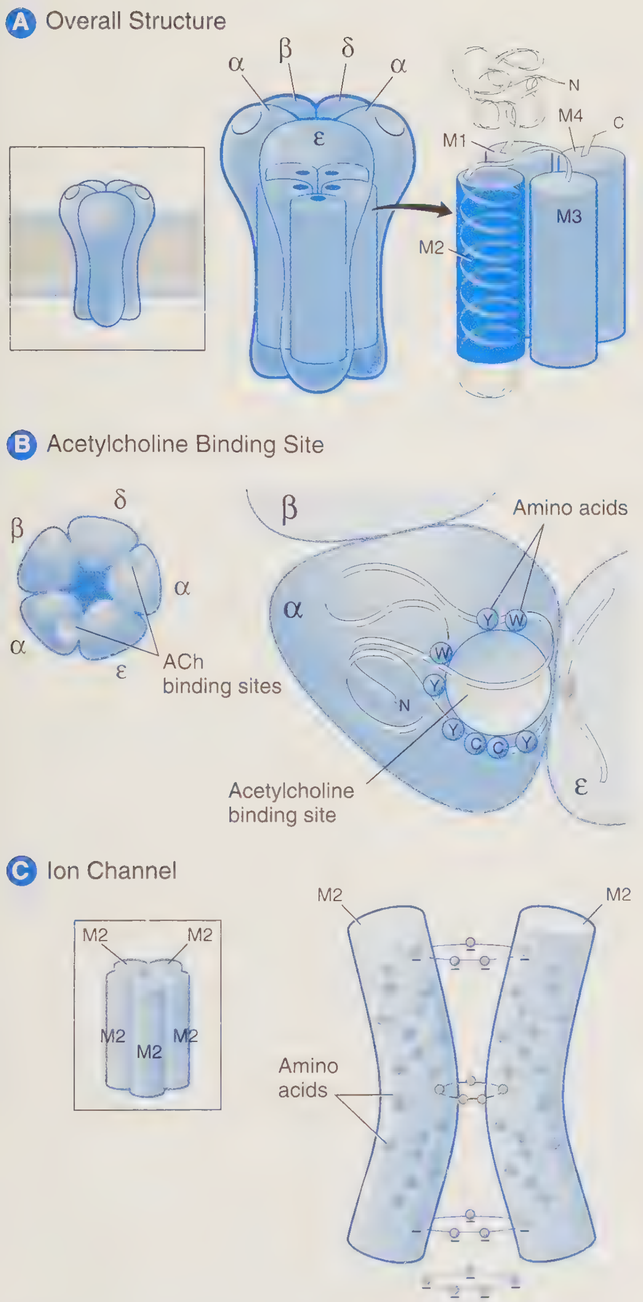


**Figure 7-2. Structural Biology of the Nicotinic Acetylcholine Receptor.** A. Overall structure of the nicotinic acetycholine receptor ($N_M$ type) and its five subunits ($\alpha_2\beta\epsilon\delta$). Each subunit is composed of a transmembrane protein that has four membrane-spanning (hydrophobic) alpha-helical regions (M1, M2, M3, M4). The large hydrophilic N-terminal domains of the two α subunits contain the binding sites for acetylcholine. B. Acetylcholine binding site viewed from above (inset: lower magnification). The labeled amino acids of the α subunit hydrophilic domain are particularly important in binding acetylcholine. The conformational change that results from the binding of two acetylcholine molecules opens the channel. C. The M2 domains of the five subunits all face the interior of the protein, and together form the transmembrane channel (inset). Three negatively charged rings of five amino acids (one from each M2 subunit) draw positively charged ions through the channel. At the center, an uncharged leucine ring (black) participates in closing the ion channel when the receptor becomes desensitized to acetylcholine.

tal muscle, while the $\alpha_2\beta\gamma\delta$ form is found in embryonic muscle.) The α *subunits are responsible for binding ACh*—this is the structural basis for the binding of two ACh molecules per receptor. The conformational change in the α subunit induced by the binding of ACh is responsible for permitting ion flow through the central pore of the receptor.

Beside simply opening and closing in response to ACh stimulation, nicotinic receptors also modulate their responses to various concentration profiles of ACh. The receptors react differently to discrete, brief pulses of ACh than to neurotransmitter that is present continuously. As noted above, under normal conditions a closed, resting-state channel responds to dual ACh binding by opening transiently, and the low affinity of the receptor for ACh causes rapid dissociation of ACh from the receptor and resumption of the receptor's resting state configuration. On the other hand, continuous exposure of the receptor to ACh causes it to undergo a change to a "desensitized" conformation in which the channel is locked closed. The desensitized state is also characterized by a greatly increased affinity of the receptor for ACh, so that ACh remains bound to the receptor for a relatively long period of time. This prolonged binding of ACh to the desensitized conformation of the receptor delays the conversion of the receptor to its unstimulated, resting state.

Nicotinic cholinergic receptors at autonomic ganglia and in the central nervous system (termed $\mathbf{N_2}$ or $\mathbf{N_N}$) are similar to receptors at the neuromuscular junction (NMJ) ($\mathbf{N_1}$ or $\mathbf{N_M}$), with the exception that the subunits in $N_N$ receptors are composed solely of α and β subunits. To complicate matters, however, seven different α subunit types ($\alpha_2$–$\alpha_8$) and three β subunit types ($\beta_2$–$\beta_4$) have been detected in neuronal tissues. ($\alpha_1$ and $\beta_1$ refer to the distinct subunit types found at the NMJ.) This diversity of α and β subunit combinations is responsible for the variable responses of CNS and autonomic nAChRs to pharmacologic agents.

## DEGRADATION OF ACETYLCHOLINE

For acetylcholine to be useful for rapid, repeated transmission, there must exist a mechanism to limit the duration of action of this transmitter. Degradation of ACh is essential not only to prevent unwanted activation of neighboring neurons or muscle cells, but also to ensure proper timing of signaling to the postsynaptic cell. A single receptor molecule is typically capable of distinguishing between two sequential presynaptic release events because degradation of ACh in the synaptic cleft occurs faster than the time course of nAChR activation.

Enzymes collectively known as **cholinesterases** are responsible for degrading acetylcholine. The two types of cholinesterase, **acetylcholinesterase (AChE)** and **butyrylcholinesterase (BuChE,** also known as pseudocholinesterase), are distributed widely throughout the body. AChE is capable of hydrolyzing about $4 \times 10^5$ molecules of ACh per enzyme molecule per minute; its turnover time of 150 μs makes it one of the most efficient hydrolytic enzymes known. Inactivating mutations of BuChE have no appreciable clinical effect, indicating that AChE is indispensable for the degradation of ACh. Because of its central importance to cholinergic transmission, an entire class of drugs known as acetylcholinesterase inhibitors has been designed to target AChE.

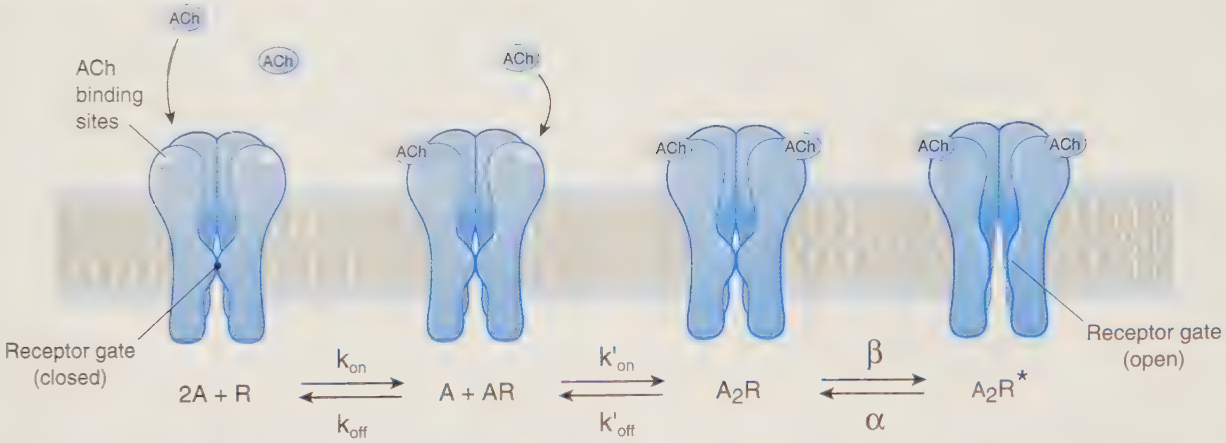

ACh
ACh binding sites
ACh
ACh
ACh
ACh
ACh
ACh
ACh
Receptor gate (closed)
Receptor gate (open)
$2A + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} A + AR \underset{k'_{off}}{\overset{k'_{on}}{\rightleftarrows}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftarrows}} A_2R^*$

**Figure 7-3. Kinetics of Nicotinic Acetylcholine Receptor Binding and Channel Opening.** Each transition between states of receptor binding and channel opening is completely reversible, and it is not necessary to go through all of the possible conformations before returning to a given state. For example, a receptor with two associated ligands may lose one and then gain another to return to its initial state, without the need for both ligands to dissociate. A = ligand (ACh), R = nicotinic ACh receptor (closed), R* = nicotinic ACh receptor (open), $k_{on}$ = rate constant for association (binding) of the first ACh molecule to the receptor, $k'_{on}$ = rate constant for association of the second ACh molecule to the receptor, $k_{off}$ = rate constant for dissociation of the first ACh molecule from the receptor, $k'_{off}$ = rate constant for dissociation of the second ACh molecule from the receptor, $\beta$ = rate constant of channel opening after both ACh molecules have bound, $\alpha$ = rate constant of channel closure. Note that channel opening and closing are much slower events than binding of ACh to the receptor.

## PHYSIOLOGIC EFFECTS OF CHOLINERGIC TRANSMISSION

### Neuromuscular Junction

Acetycholine is the principal neurotransmitter at the neuromuscular junction (Fig. 7-4). The binding of ACh released by $\alpha$ motor neurons to nicotinic receptors in the muscle cell membrane results in motor end-plate depolarization. The extent of depolarization depends on the quantity of ACh released into the synaptic cleft. It is now well established that the release of ACh is quantal in nature; that is, ACh is released in discrete quantities by the motor neuron. Each quantum of ACh corresponds to the contents of a single synaptic vesicle, and elicits a very small depolarization in the motor end-plate termed a **miniature end-plate potential (MEPP).** Under resting conditions, sporadic MEPPs are detected at the motor end-plate, corresponding to a low baseline level of unstimulated ACh release that arises from spontaneous vesicle fusion with the motor axon's presynaptic membrane. In contrast, the arrival of an action potential at the motor axon terminal causes many more vesicles (up to thousands) to fuse with the neuronal membrane and release their ACh simultaneously. At the motor end-plate, the result is a relatively large depolarization termed the **end-plate potential (EPP)** (Fig. 7-5). The magnitude of the EPP is more than sufficient to trigger a propagating action potential throughout the muscle fiber and, hence, to produce a single contraction or "twitch."

*Acetylcholine not only triggers muscle contraction as its primary effect at the NMJ, but it also modulates its own action at this site.* Presynaptic cholinergic receptors, located on the axon terminal of the motor neuron, respond to ACh by *facilitating* the mobilization of synaptic vesicles from the reserve pool to the depot pool. This positive feedback loop, through which the release of ACh encourages additional release, is necessary to ensure sufficient ACh release under high frequency stimulation of the nerve (approximately 100 Hz). Despite this mechanism, the ACh output per nerve impulse wanes rapidly during persistent high-frequency stimulation. Fortunately, because an excess amount of ACh is released, and an excess number of ACh receptors is present, there is a large safety margin. Only when 50% or more of the postsynaptic receptors are desensitized is a decline in muscle tension observed during tetanic stimulation (a phenomenon known as **tetanic fade**). Importantly, selective blockade of the modulatory presynaptic cholinergic receptors by such antagonists as **hexamethonium** prevents facilitation and causes a rapid tetanic fade to be observed under otherwise normal conditions (Fig. 7-6).

### Autonomic Effects

Neurotransmission through autonomic ganglia is complicated by the fact that several distinct receptor types mediate the complex changes in membrane potential observed in postganglionic neurons. The generalized postsynaptic response to presynaptic impulses can be separated into four distinct components (Fig. 7-7). *The primary event in the postsynaptic ganglionic response is a rapid depolarization mediated by nicotinic ACh receptors.* The mechanism is similar to that in the NMJ, in that an inward current elicits a near-immediate **excitatory postsynaptic potential (EPSP)** of 10–50 ms duration. Typically, the amplitude of such an EPSP is only a few millivolts, and many such events must sum for the postsynaptic cell membrane to reach the threshold for firing an action potential (Fig. 7-7A). The three remaining events of ganglionic transmission serve either to amplify or suppress this primary signal, and are known as the slow EPSP, the IPSP (inhibitory postsynaptic potential), and the late, slow EPSP. The **slow EPSP** occurs after a latency of 1 second and is mediated by $M_1$ muscarinic ACh receptors. The duration of this effect is 10–30 seconds (Fig. 7-7C). The **IPSP** is largely a

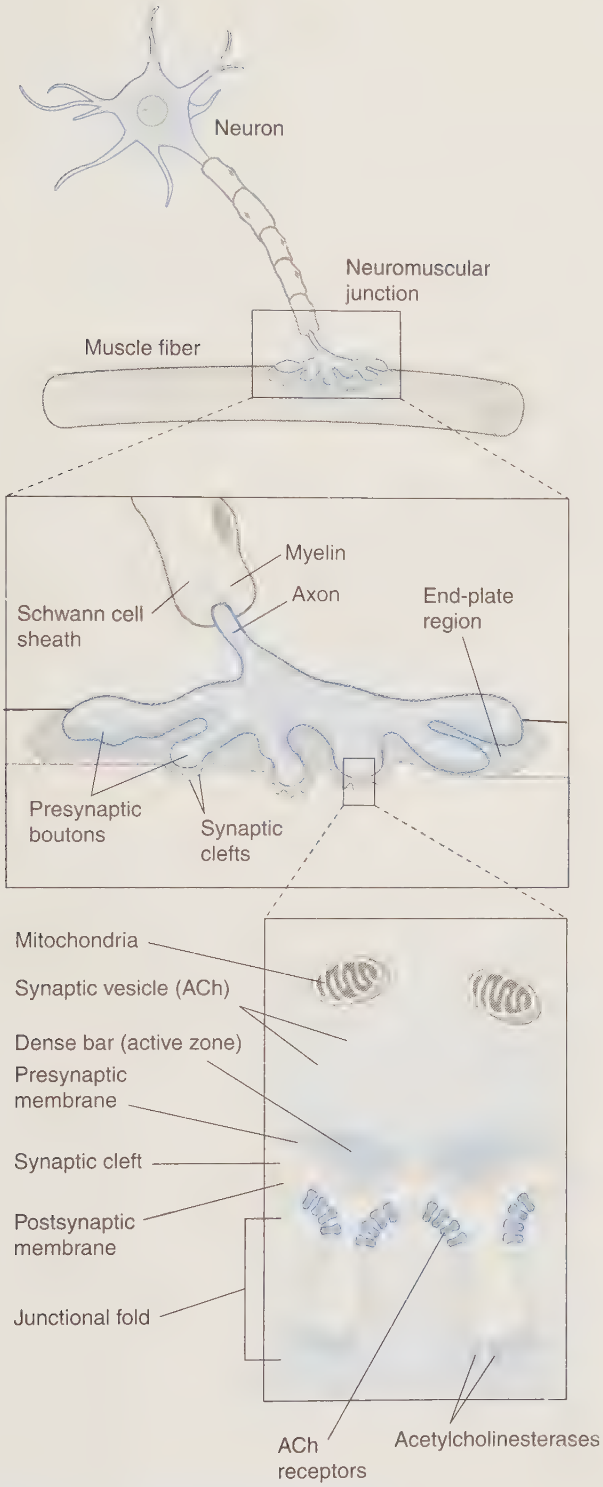


**Figure 7-4. The Neuromuscular Junction.** At the neuromuscular junction, motor neurons innervate a group of muscle fibers. The area of muscle fibers innervated by an individual motor neuron is referred to as the **end-plate** region. Multiple presynaptic terminals extend from the axon of the motor neuron. When the motor neuron is depolarized, its synaptic vesicles fuse with the presynaptic membrane, releasing ACh into the synaptic cleft. ACh receptors of the neuromuscular junction are exclusively nicotinic, and stimulation of these receptors results in depolarization of the muscle cell membrane and generation of an end-plate potential.

product of catecholamine (i.e., dopamine and norepinephrine) stimulation of dopaminergic and α-adrenergic receptors (see Chapter 8), although some IPSPs in a few ganglia are mediated by $M_2$ muscarinic receptors. The latency and duration of the IPSP generally vary between those of the fast and slow EPSPs. The **late, slow EPSP** is caused by a decrease in potassium conductance induced by stimulation of receptors for peptide transmitters (i.e., angiotensin, substance P, and luteinizing hormone-releasing hormone). Lasting for several minutes, the late, slow EPSP is thought to play a role in the long-term regulation of postsynaptic neuron sensitivity to repetitive depolarization.

One pharmacologic consequence of such a complex pattern of depolarization in autonomic ganglia is that drugs selective for the IPSP, slow EPSP, and late, slow EPSP are generally not capable of eliminating ganglionic transmission. Instead, such agents only alter the efficiency of transmission. For example, **methacholine,** a muscarinic receptor agonist, has modulatory effects on autonomic ganglia that resemble the stimulation of slow EPSPs (see below).

*The overall effect of ganglionic blockade is complex, and depends on the relative predominance of sympathetic and parasympathetic tone at the various end-organs* (Table 7-2). For example, the heart is influenced at rest primarily by the parasympathetic system. Thus, blockade of autonomic ganglia that innervate the heart results in an elevation of sympathetic tone, and hence in tachycardia. Blood vessels, in contrast, are innervated only by the sympathetic system. Because the normal effect of sympathetic stimulation is to cause vasoconstriction, ganglionic blockade results in vasodilation. It is important to realize, however, that the responses described above ignore the presence of muscarinic ACh receptors at many of the end organs. When stimulated directly by cholinergic agents, such receptors often mediate a response that overrides the response produced by ganglionic blockade.

The muscarinic receptors found in visceral smooth muscle, cardiac muscle, secretory glands, and endothelial cells mediate highly diverse responses to cholinergic stimulation. These effects are detailed in Table 7-3. In general, these end-organ effects tend to predominate over ganglionic influences. That is, for systemically administered cholinergic agents, the overall response is generally similar to that caused by direct stimulation of these postganglionic effector sites, and often different from that caused by ganglionic stimulation.

## CNS Effects

CNS functions that are currently attributed to ACh include suppression of pain at the spinal cord level, sleep and wakefulness, attention, early neural development, and immunosuppression. Although research is ongoing in all of these fields, and current understanding of the roles of cholinergic transmission in the CNS far surpasses that of a decade ago, many functions and details are still unknown.

Acetylcholine is thought to play a role in pain modulation through inhibition of spinal nociceptive transmission. Cholinergic neurons located in the rostral ventromedial medulla extend processes to the superficial lamina of the dorsal horn at all levels of the spinal cord, where secondary neurons in afferent sensory pathways are located. ACh released by the cholinergic neurons is believed to bind to muscarinic ACh receptors located on secondary sensory neurons specific for pain,

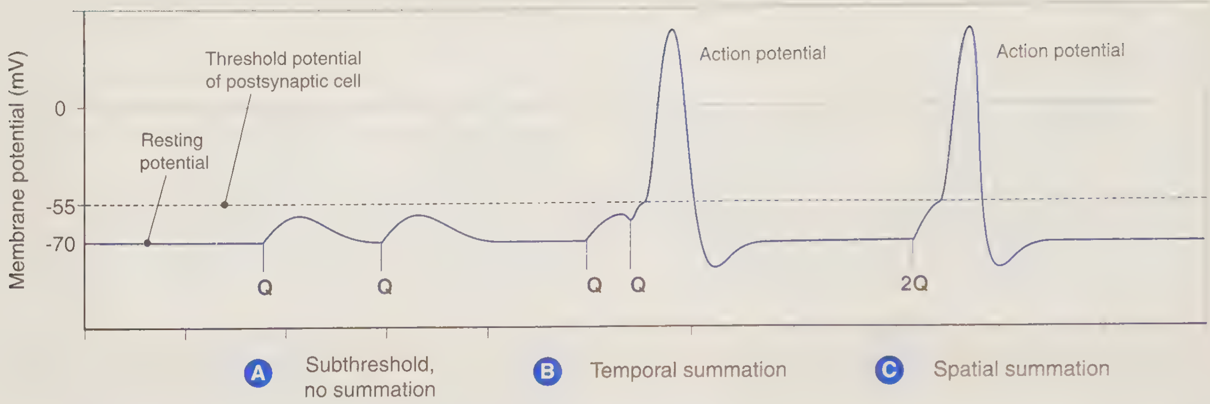


**Figure 7-5. Quantal Release of Acetylcholine and Muscle Contraction.** Muscle contraction relies on the accumulation of a sufficient concentration of acetylcholine at the motor end-plate to depolarize the muscle beyond the threshold potential (typically about −55 mV). After local depolarization occurs, a self-propagating action potential is generated that can spread along the muscle fiber and result in muscle contraction. **A.** As a single cholinergic vesicle releases its contents into the neuromuscular junction (NMJ), a small depolarization (Q), otherwise known as a miniature end-plate potential (MEPP), occurs in the local region of the muscle. This MEPP is insufficient to generate an action potential. When a sufficient number of individual cholinergic vesicles empty their contents into the NMJ, either in quick succession **(B)** or simultaneously **(C)**, sufficient depolarization occurs (termed the end-plate potential, or EPP) that the motor end-plate threshold for action potential generation is exceeded, and muscle contraction occurs. An isolated action potential produces a twitch, while a train of action potentials may produce sustained contraction of the muscle. Note that, although this example uses two MEPPs for simplicity, many more than two MEPPs are actually required to achieve threshold-level depolarization.

resulting in suppression of action potential firing in these cells and consequently in analgesia (see Chapter 15). Clinically, the analgesic properties of ACh can be demonstrated by injecting acetylcholinesterase inhibitors into the spinal fluid.

As part of the ascending reticular activating system, cholinergic neurons play an important role in arousal and attention (Fig. 6-8). Levels of ACh throughout the brain increase during wakefulness and REM sleep and decrease during inattentive states and non-REM sleep. There is strong evidence that depletion of cortical ACh results in markedly decreased performance in tasks requiring sustained, selective, and divided attention. In contrast, repetitive tasks do not seem to depend on the activity of cholinergic neurons, unless a strong motivation is given for optimal performance. (The urgency of motivation also stimulates the locus ceruleus, which releases norepinephrine and activates adrenergic neurons via $\alpha_1$ receptors.)

During an awake or aroused state, cholinergic projections from the pedunculopontine nucleus, the lateral tegmental nucleus, and the nucleus basalis of Meynert (NBM) are all active. Because the NBM extends processes diffusely throughout the entire cortex and hippocampus (Fig. 6-8), activation of the NBM causes a global increase in ACh levels, which is thought to have effects on brain activity that promote attention. ACh markedly potentiates the excitatory effects of other inputs to its cortical target cells, without affecting the baseline activity of these neurons. This primed state is thought to improve the ability of such neurons to process incoming inputs. For the brain as a whole, the result is a heightened state of responsiveness.

The importance of ACh for cognitive function is illustrated by the pathophysiology of **Alzheimer's disease** (AD). Patients with this disease have *significant loss of cholinergic neurons.* Although the loss occurs throughout the CNS, the most severely affected regions are in the temporal lobe and entorhinal cortex, where more than 75% of cholinergic neurons are destroyed. Interestingly, these areas of greatest cholinergic deficiency are also the areas responsible for maintaining the functions that are most impaired in AD, including memory and cognition. Further evidence for the potential role of ACh in AD is that several anticholinesterases seem to be modestly effective in ameliorating symptoms associated with AD.

Recent studies also suggest that ACh may have CNS effects unrelated to its role as a neurotransmitter. ACh has been observed to inhibit neurite growth. During the early phases of neural development, when such growth is essential, AChE levels are increased. The presence of ACh in chick limb buds and myotomes suggests other, morphogenetic roles for this compound. Lesioning of rat cholinergic neurons during development results in cortical abnormalities, including aberrant growth and positioning of pyramidal cell dendrites, altered cortical connectivity, and gross cognitive defects. These abnormal findings are observed in fetal alcohol syndrome and Rett syndrome, both of which demonstrate dramatically reduced numbers of cholinergic neurons in the brain. There is also some evidence for an immunomodulatory role for ACh, as many cells of the immune system both release ACh and possess ACh receptors.

## PHARMACOLOGIC CLASSES AND AGENTS

Some years ago, there was considerable interest in developing pharmacologic agents to take advantage of the central role that ACh plays in numerous physiologic pathways. The complex and diffuse actions of ACh have made it difficult to produce selective effects using modulation of cholinergic pathways,

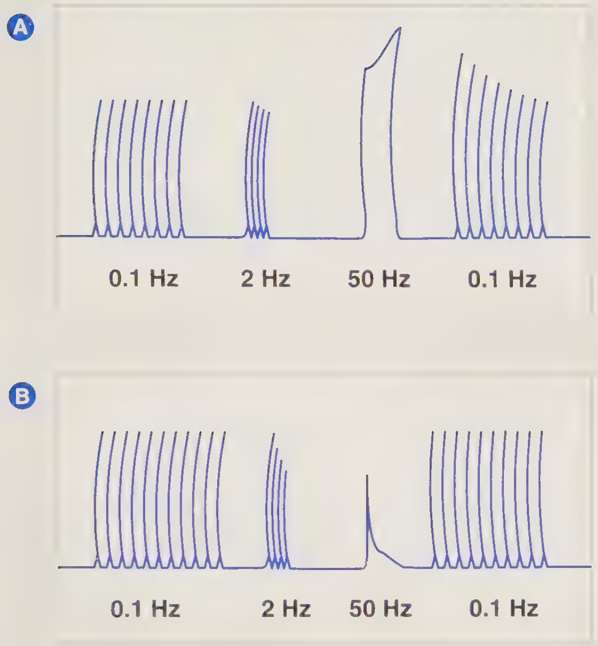


**Figure 7-6. Tetanic Fade and the Effects of Hexamethonium.** **A.** Control stimulation. Rapid stimulation of muscle contraction relies upon presynaptic acetylcholine autoreceptors that provide positive feedback and, thereby, increase the amount of acetylcholine released with each depolarization. The diagram shows control muscle responses to single shock stimulation (0.1 Hz), a train-of-four stimulations (2 Hz), or tetanic stimulation (50 Hz). Positive feedback increases the amount of ACh released with each depolarization during tetanic stimulation, providing enhanced muscle contraction that gradually fades back to baseline during subsequent single shock stimulation. **B.** Stimulation after the administration of hexamethonium. Note that, although the response to isolated (0.1 Hz), stimuli is unchanged in the presence of hexamethonium, the drug prevents the increased effect that normally occurs with higher-frequency (50 Hz) stimulation. This is a result of hexamethonium's antagonism of the acetylcholine autoreceptor on the presynaptic terminal that is normally responsible for positive feedback of ACh release.

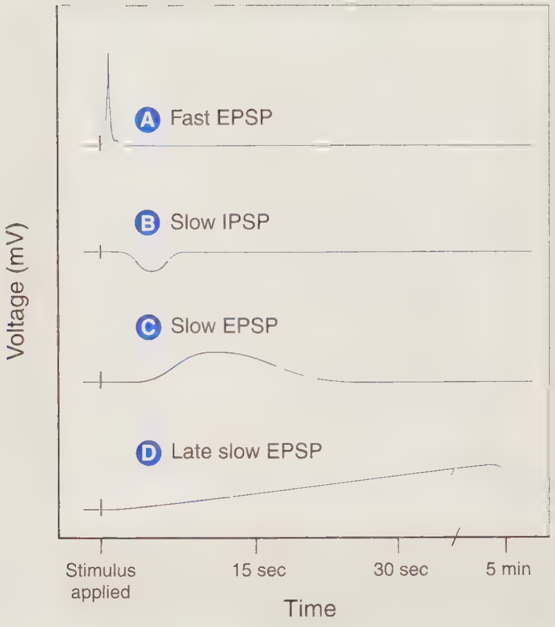


**Figure 7-7. Four Types of Synaptic Signals in an Autonomic Ganglion.** The response of autonomic ganglia to neurotransmission is a complex event mediated by a number of different neurotransmitters and receptor types, and occurring on several distinct time scales. **A.** The primary mode of neurotransmission is the action potential, which is produced by a sufficiently strong (suprathreshold) excitatory postsynaptic potential (EPSP). The fast EPSP is mediated by acetylcholine acting on postsynaptic nicotinic ACh receptors. **B.** The slow inhibitory postsynaptic potential (IPSP) is a membrane hyperpolarization response. This response is thought to be mediated by several different postsynaptic receptor types, including modulatory dopamine receptors and $\alpha$-adrenergic receptors as well as $M_2$ muscarinic ACh receptors. **C.** The slow EPSP is mediated by $M_1$ muscarinic receptors, has a latency of about 1 second after an initial depolarization, and lasts for 10–30 seconds. **D.** The late, slow EPSP occurs on the order of minutes after a depolarization event. This excitatory response may be mediated by peptides that are coreleased with acetylcholine.

however. This problem has been complicated further by the fact that many cholinergic agents are capable of both stimulating and blocking cholinergic receptors through a process known as depolarizing blockade (see below). As a result, the therapeutic uses of cholinergic drugs remain limited. Of the many cholinergic and anticholinergic agents discovered over the past century, only a handful see clinical application. These drugs are used primarily for (1) modulation of GI motility, (2) xerostomia (dry mouth), (3) glaucoma, (4) motion sickness, (5) such neuromuscular diseases as myasthenia gravis and Eaton–Lambert syndrome, (6) acute neuromuscular blocking and reversing, (7) ganglionic blocking during aortic dissection and (8) Alzheimer's disease.

Slight variations in the pharmacologic properties of individual cholinergic and anticholinergic agents are responsible for their rather large differences in therapeutic utility. The relative selectivity of action of the most useful agents depends on both pharmacodynamic and pharmacokinetic factors, including inherent differences in receptor binding affinity, bioavailability, tissue localization, and resistance to degradation. These variations, in turn, derive from the molecular structure and charge of the agent. The structure of **pirenzepine,** for example, allows the drug to bind $M_1$ muscarinic receptors (located in autonomic ganglia) with higher affinity than $M_2$ and $M_3$ receptors (located at parasympathetic end organs). As a result, the drug's predominant effect at clinically used doses is ganglionic blockade (see Table 7-1). Similarly, the addition of a methyl group to acetylcholine yields **methacholine,** which is more resistant to degradation by AChE and, hence, possesses a longer duration of action. Such charged agents as muscarine, generally have difficulty crossing membrane barriers. The absorption of such drugs through both the GI mucosa and the blood–brain barrier is significantly impaired, unless specific carriers are available to transport the drug; therefore, such drugs usually have little to no effect on the CNS. In contrast, lipophilic agents have excellent CNS

TABLE 7-2 Effects of Autonomic Ganglionic Blockade on Tissues

| SITE | PREDOMINANT TONE | EFFECTS OF GANGLIONIC BLOCKADE |
|---|---|---|
| Arterioles | Sympathetic (adrenergic) | Vasodilation; ↑ peripheral blood flow; hypotension |
| Veins | Sympathetic (adrenergic) | Vasodilation; pooling of blood; ↓ venous return; ↓ cardiac output |
| Heart | Parasympathetic (cholinergic) | Tachycardia |
| Iris | Parasympathetic (cholinergic) | Mydriasis (pupil dilation) |
| Ciliary muscle | Parasympathetic (cholinergic) | Cycloplegia (focused to far vision) |
| Gastrointestinal tract | Parasympathetic (cholinergic) | ↓ Tone and motility; constipation; ↓ secretions |
| Urinary bladder | Parasympathetic (cholinergic) | Urinary retention |
| Salivary glands | Parasympathetic (cholinergic) | Xerostomia (dry mouth) |
| Sweat glands | Sympathetic (cholinergic) | Anhidrosis (absence of sweating) |

penetration. As one example, the high CNS penetration of **physostigmine** makes this drug the agent of choice for treating the CNS effects of anticholinergic overdose.

The following discussion is ordered mechanistically by the site of action of the various classes of agents in cholinergic pathways. For each class of drugs, the selectivity of individual agents within the class is used as a basis to explain the therapeutic uses, if any, of each drug.

## INHIBITORS OF ACETYLCHOLINE SYNTHESIS, STORAGE, AND RELEASE

Drugs that inhibit the synthesis, storage, or release of ACh have only recently begun to have clinical use (Fig. 7-1). **Hemicholinium-3** blocks the high-affinity transporter for choline and, thus, prevents the uptake of choline required for ACh synthesis. **Vesamicol** blocks the ACh-$H^+$ antiporter that is used to transport ACh into vesicles, thereby preventing the storage of ACh. Both of these compounds are utilized only in research settings, however. **Botulinum toxin,** produced by *Clostridium botulinum,* degrades synaptobrevin and, thus, prevents synaptic vesicle fusion with the axon terminal membrane. This paralysis-inducing property is currently used in the treatment of several diseases associated with increased muscle tone, such as achalasia, strabismus, and blepharospasm. Botulinum toxin has also recently been approved for cosmetic treatment of facial lines or wrinkles.

## ACETYLCHOLINESTERASE INHIBITORS

Agents in this class bind to and inhibit acetylcholinesterase, thereby elevating the concentration of endogenously released ACh in the synaptic cleft. The excess ACh subsequently activates nearby cholinergic receptors. Agents in this class are also referred to as indirectly acting ACh receptor agonists because they do not activate receptors directly. It is important to note that a few indirectly acting agents have a direct action as well. For example, **neostigmine,** a quaternary carbamate, not only blocks cholinesterase, but also binds to and activates nAChRs at the neuromuscular junction.

TABLE 7-3 Effects of Acetylcholine on Peripheral Tissues

| TISSUE | EFFECTS OF ACETYLCHOLINE |
|---|---|
| Vasculature (endothelial cells) | Release of nitric oxide and vasodilation |
| Eye iris (pupillae sphincter muscle) | Contraction and miosis |
| Ciliary muscle | Contraction and accommodation of lens to near vision |
| Salivary and lacrimal glands | Thin and watery secretions |
| Bronchi | Constriction; ↑ secretions |
| Heart | Bradycardia, ↓ conduction velocity, AV block at high doses, slight ↓ in contractility |
| Gastrointestinal tract | ↑ Tone, secretions; relaxation of sphincters |
| Urinary bladder | Contraction of detrusor muscle; relaxation of sphincter |
| Sweat glands | Diaphoresis |
| Reproductive tract, male | Erection |
| Uterus | Variable |

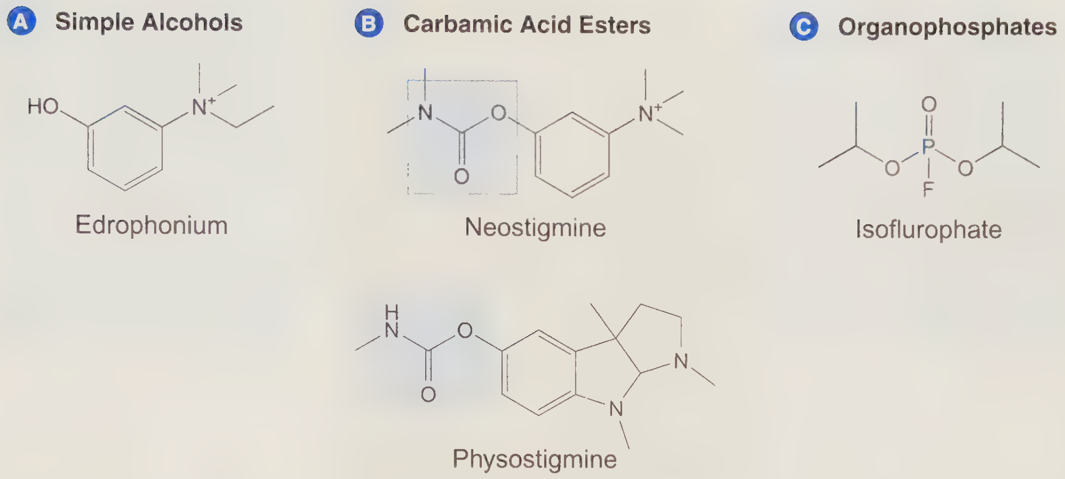


**Figure 7-8. Structural Classes of Acetylcholinesterase Inhibitors.** Acetylcholinesterase (AChE) inhibitors are divided into three structural classes. **A.** Such simple alcohols as edrophonium have a short half-time of AChE inhibition. Edrophonium is used in the diagnosis of myasthenia gravis and other diseases of the neuromuscular junction. **B.** Carbamic acid esters are hydrolyzed by AChE. This results in the formation of a covalent bond between the carbamic acid ester (boxed) and AChE, and a consequently long half-time of AChE inhibition. Neostigmine is used to treat myasthenia gravis and, during or after surgery, to reverse paralysis induced by nicotinic acetylcholine receptor antagonists. Physostigmine, because it has good CNS penetration, is the agent of choice for treating anticholinergic poisoning. **C.** Organophosphates form an extremely stable phosphorus-carbon bond with AChE. This results in irreversible inactivation of AChE. As a result, many organophosphates are extremely toxic.

## Structural Classes

All indirectly acting cholinergic agonists interfere with the functioning of AChE by binding to the active site of the enzyme. There are three chemical classes of such agents, including (1) simple alcohols with a quaternary ammonium group, (2) carbamic acid esters of alcohols bearing either quaternary or tertiary ammonium groups, and (3) organic derivatives of phosphoric acid (Fig. 7-8). The most important functional difference among these classes is in their pharmacokinetics.

**Edrophonium** is a simple alcohol that inhibits AChE by reversibly associating with its active site. Because of the noncovalent nature of the interaction between the alcohol and AChE, the enzyme–inhibitor complex lasts for only 2–10 minutes, resulting in a relatively rapid and completely reversible block.

The carbamic acid esters **neostigmine** and **physostigmine** are actually hydrolyzed by AChE, so a labile covalent bond is formed between the drug and the enzyme. However, *the rate at which this reaction occurs is many orders of magnitude slower than for ACh.* The resulting enzyme-inhibitor complex has a half-life of approximately 15–30 minutes, corresponding to an effective inhibition lasting 3–7 hours in vivo.

Such organophosphates as **diisopropyl fluorophosphate** have a molecular structure that resembles the transition state formed in carboxyl ester hydrolysis. These compounds are hydrolyzed by AChE, but the resulting phosphorylated enzyme complex is extremely stable and dissociates with a half-life of hundreds of hours. Furthermore, the enzyme–organophosphate complex is subject to a process known as aging, in which oxygen–phosphorous bonds within the inhibitor are broken spontaneously in favor of stronger bonds between the enzyme and the inhibitor (see also Chapter 47). Once aging occurs, the duration of AChE inhibition is increased even further. Thus, prolonged organophosphate inhibition is essentially irreversible, and the body must synthesize new AChE molecules to restore AChE activity. However, if strong nucleophiles (such as **pralidoxime**) are administered before aging has occurred, it is possible to recover AChE function.

## Clinical Applications

Acetylcholinesterase inhibitors have a broader spectrum of clinical uses than do directly acting cholinergic agonists, largely because of the pharmacokinetic variation that is available among the different agents. These applications include (1) increasing transmission at the neuromuscular junction, (2) increasing parasympathetic tone, and (3) increasing cholinergic activity in the CNS in Alzheimer's disease.

Because of their ability to increase the activity of endogenous ACh, anticholinesterases are especially useful in diseases of the neuromuscular junction, where the primary defect is an insufficient quantity of either ACh or AChR. In myasthenia gravis, autoantibodies are generated against $N_M$ receptors. These antibodies both induce $N_M$ receptor internalization and block the ability of ACh to activate the receptors. As a result, patients with myasthenia gravis present with significant weakness (recall the description of Chief Opechancanough in the introductory case). Eaton–Lambert syndrome is also characterized by muscle weakness, but this disorder is caused by autoantibodies generated against $Ca^{2+}$ channels; both presynaptic $Ca^{2+}$ entry and the subsequent release of ACh in response to axon terminal depolarization are attenuated. Certain anticholinergic drugs (such as tubocurare) also

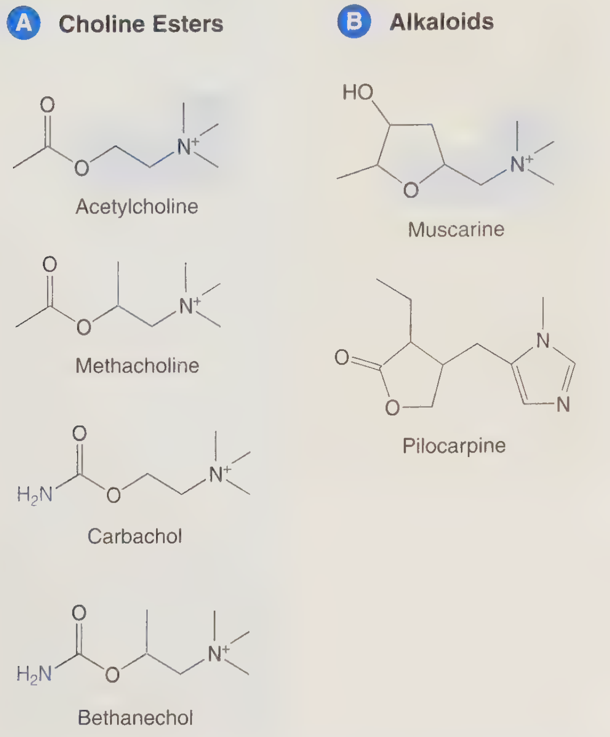


Figure 7-9. Structural Classes of Muscarinic Receptor Agonists. Muscarinic receptor agonists are divided into choline esters and alkaloids. **A.** Choline esters are all charged molecules and; therefore, have little CNS penetration. Methacholine is highly resistant to AChE and is used in the diagnosis of asthma. Carbachol has both nicotinic and muscarinic receptor activity; it is only used topically for treatment of glaucoma. Bethanechol is highly selective for muscarinic receptors; it is used to promote gastrointestinal and bladder motility. **B.** Alkaloids have highly variable structures; some have excellent CNS penetration. Muscarine, the prototypical muscarinic receptor agonist, is an alkaloid that is structurally similar to acetylcholine (boxed areas). Pilocarpine is the only alkaloid muscarinic receptor agonist that is used clinically. It is used to treat xerostomia (dry mouth) in patients with Sjogren's syndrome.

cause weakness, or even paralysis, by acting as competitive antagonists at the nAChR. Acetylcholinesterase inhibitors (such as the physostigmine used in the introductory case) improve all three of these conditions, by increasing the concentration of endogenously released ACh at the neuromuscular junction and, thereby, increasing the number of ACh-bound receptors.

Because the binding of ACh to $N_M$ receptors results in muscle depolarization, *AChE inhibitors are ineffective at reversing the action of agents that cause paralysis by inducing sustained depolarization, such as succinylcholine* (see below). In fact, anticholinesterases in sufficiently high doses can exacerbate existing weakness and paralysis because of depolarizing blockade. Thus, it is of fundamental importance that the nature of the muscle weakness is examined carefully before treatment is initiated. Such short-acting anticholinesterases as edrophonium are ideal for such diagnostic purposes. **Edrophonium** will mitigate weakness if the blockade is attributable to competitive AChR antagonists or to such diseases as myasthenia gravis or Eaton–Lambert syndrome. In contrast, if muscle strength decreases further with edrophonium administration, then depolarizing blockade may be suspected. The short half-life of edrophonium ensures that exacerbation of the latter condition will last for a minimal amount of time. For chronic treatment of myasthenia gravis, longer-acting anticholinesterases such as **pyridostigmine, neostigmine** and **ambenonium** are the preferred agents.

AChE inhibitors mediate other therapeutic effects by potentiating parasympathetic actions in target tissues. Topical application of anticholinesterases to the cornea of the eye decreases intraocular pressure by facilitating the outflow of aqueous humor. The main effect of AChE inhibitors on the gastrointestinal system is an increase in smooth muscle motility because of enhancement of ganglionic transmission at Auerbach's plexus, although these agents also cause increased secretion of gastric acid and saliva. Neostigmine, the most popular drug for this application, is typically used for relief of abdominal distention. The use of anticholinesterases in reversing anticholinergic drug poisoning is also well documented. The agent of choice for this indication is typically **physostigmine;** its tertiary amine structure allows it ready access to the brain and spinal cord, where it can counteract the CNS effects of anticholinergic toxicity.

Several studies have demonstrated a deficiency of cholinergic neurons, particularly those belonging to the nucleus basalis of Meynert, in patients with Alzheimer's disease. There are currently four AChE inhibitors—**tacrine, donepezil, rivastigmine,** and **galantamine**—approved by the FDA for use in the treatment of this disease. All of these agents delay the development of cognitive and functional impairment by approximately 6 months. None of the drugs has a demonstrated effect on disease-related mortality, however, and none ultimately prevent the progressive degenerative course of Alzheimer's. Tacrine is rarely used, because of concerns regarding potential hepatotoxicity. All of these agents can cause gastrointestinal distress.

## RECEPTOR AGONISTS

All cholinergic receptor agonists bind to the ACh-binding site of cholinergic receptors. Receptor agonists can be divided roughly into muscarinic and nicotinic receptor-selective agents, although some cross reactivity exists with virtually all of these agents. Muscarinic receptor agonists can be used clinically in the diagnosis of asthma and as miotics (agents that cause pupil constriction). The only clinically useful nicotinic receptor agonist is **succinylcholine,** which is used to induce depolarizing blockade of the NMJ and subsequent muscle paralysis.

### Muscarinic Receptor Agonists

Agents in this class are divided structurally into choline esters and alkaloids (Fig. 7-9). The choline esters are all charged, highly hydrophilic molecules that are poorly absorbed by the oral route and ineffectively distributed to the CNS. Choline esters include acetylcholine, methacholine, carbachol, and bethanechol (Table 7-4). Acetylcholine is not

**TABLE 7-4** Relative Pharmacologic Properties of Choline Esters

| ESTER | SUSCEPTIBILITY TO AChE | CARDIAC ACTIVITY | GI ACTIVITY | URINARY ACTIVITY | EYE ACTIVITY (TOPICAL) | ATROPINE ANTAGONISM | NICOTINIC ACTIVITY |
|---|---|---|---|---|---|---|---|
| Acetylcholine | +++ | ++ | ++ | ++ | + | +++ | ++ |
| Methacholine | + | +++ | ++ | ++ | + | +++ | + |
| Carbachol | – | + | +++ | +++ | ++ | + | +++ |
| Bethanechol | – | ± | +++ | +++ | ++ | +++ | – |

Note that all actions are mediated by muscarinic receptors, with the exception of nicotinic activity. "–": negligible activity. "±": unpredictable

administered in clinical settings because of both its broad actions and its extremely rapid hydrolysis by AChE and pseudocholinesterase.

**Methacholine** is at least three times more resistant to hydrolysis than ACh. This agent is relatively selective for cardiovascular muscarinic cholinergic receptors, and it possesses relatively little affinity for nicotinic cholinergic receptors. Although methacholine can stimulate receptors found on cardiovascular tissue, the magnitude of its response is unpredictable. This fact has limited its use as a vasodilator or cardiac **vagomimetic** [i.e., a drug that mimics the cardiac response to vagus nerve (parasympathetic) stimulation, which typically involves bradycardia, decreased contractility, and compensatory sympathetic reflexes]. Currently, methacholine is used only in the diagnosis of asthma because the bronchial hyperreactivity that is characteristic of asthma causes an exaggerated bronchoconstriction response to parasympathomimetics (see Chapter 45).

Both carbachol and bethanechol are resistant to cholinesterases because, in these drugs, a carbamoyl group is substituted for the acetyl group of ACh (Fig. 7-9). This resistance to AChE extends their duration of action and allows time for distribution to areas of lower blood flow. **Carbachol** has enhanced nicotinic action relative to other choline esters. This agent cannot be used systemically because its nicotinic action at autonomic ganglia leads to unpredictable responses. Instead, the agent is used principally as a miotic agent, typically in the treatment of glaucoma. Topical application of the drug to the cornea of the eye results in both pupil constriction (miosis) and decreased intraocular pressure, without the unpredictable side effects that would otherwise occur with systemic administration.

**Bethanechol** is almost completely selective for muscarinic receptors. It is the agent of choice for promoting GI and urinary tract motility.

In contrast to the choline esters, the alkaloids vary greatly in structure. Some are amphipathic; whereas, others are highly charged. Most of these agents are tertiary amines, although a few are quaternary amines with protonated or permanently charged nitrogens substituting for the choline-centered N of ACh. The amphipathic nature of the tertiary amine alkaloids permits absorption through the GI mucosa and penetration into the CNS. Muscarine is an example of a quaternary amine alkaloid that has poorer bioavailability because of its permanently charged nature.

Most alkaloids are primarily of value in pharmacologic research. The most significant clinically used alkaloid is **pilocarpine,** which is used primarily as a miotic agent and as a sialagogue (saliva-inducing agent) to combat xerostomia (dryness of the mouth secondary to reduced salivary secretion).

## Nicotinic Receptor Agonists

**Succinylcholine** is a choline ester that has a high affinity for nicotinic receptors and is resistant to AChE. It is used to induce paralysis during surgery by means of **depolarizing blockade.** This effect can be caused by any direct nAChR *agonist* because such drugs activate cholinergic channels and produce depolarization of the cell membrane. To produce depolarizing blockade, the agent must persist at the neuroeffector junction and activate the nicotinic receptor channels continually. (Note that this effect is unlike the depolarization pattern seen in the generation of a standard action potential or end-plate potential, in which ACh is present at the neuroeffector junction for only a brief period of time.) The result is a brief period of excitation, manifested by widespread fasciculations in muscle cells, followed by flaccid paralysis. The paralysis occurs because the open cholinergic channels maintain the cell membrane in a depolarized condition, effecting inactivation of voltage-gated sodium channels so that they cannot open to support any further action potentials. Because of this mechanism, *any nAChR agonist, including ACh, is capable of producing depolarizing blockade at sufficiently high concentrations.* Generally, depolarizing blockade with succinylcholine is used for only short durations because prolonged depolarization can lead to life-threatening electrolyte imbalances (caused by prolonged $Na^+$ influx and $K^+$ efflux). Table 7-5 compares the effects of depolarizing and nondepolarizing NMJ-blocking agents (discussed below).

The concept of depolarizing blockade pertains to *all* cholinergic receptors, and is *not strictly limited to the NMJ.* For example, this mechanism accounts for the paradoxical suppression of parasympathomimetic activity at autonomic ganglia by high levels of agonists, such as nicotine, that are selective for nicotinic receptors. The potential for inducing depolarizing blockade is partially responsible for the unpredictable effects of nAChR agonists. Although muscarinic receptor agonists can also cause depolarizing blockade at autonomic ganglia, this effect is obscured by the overwhelmingly parasympathomimetic responses seen at other neuroeffector sites.

**TABLE 7-5** Comparison of Competitive and Depolarizing NMJ-Blocking Agents

| | COMPETITIVE | DEPOLARIZING |
|---|---|---|
| Previous administration of a competitive NMJ-blocking agent | Additive | Antagonistic effect |
| Previous administration of a depolarizing NMJ-blocking agent | No effect or antagonistic | No effect or additive |
| Effect on motor end-plate | Elevated activation threshold to ACh; no depolarization | Partial; persisting depolarization |
| Initial excitatory effect on muscle | None | Transient fasciculations |
| Muscle response to tetanic stimulation during partial block | Poorly sustained contraction | Well-sustained contraction |

## RECEPTOR ANTAGONISTS

Antagonists of AChRs act by binding directly to the agonist site and competitively blocking stimulation of the receptor by endogenous ACh or exogenously administered receptor agonists. AChR antagonists are generally more effective at blocking cholinergic activity caused by exogenously administered agents than by endogenous ACh. This difference results from the close anatomical proximity of cholinergic nerve terminals to their target receptors, which causes the concentration of endogenously released ACh to be extremely high within the neuroeffector junction. Therefore, it is relatively more difficult to overcome competitively the very high concentrations of endogenous ACh than the lower concentrations of systemically administered cholinergic agonists.

### Muscarinic Receptor Antagonists

Anticholinergic compounds that act on muscarinic receptors are used to produce a parasympatholytic effect in target organs. By blocking normal cholinergic tone, these compounds cause sympathetic responses to predominate (Table 7-2). Chemically, the most commonly encountered anticholinergics are either naturally occurring alkaloids or synthetic quaternary ammonium compounds. The alkaloids are relatively selective for antagonist activity at muscarinic receptors, while the synthetic compounds also demonstrate significant antagonism at nicotinic receptors.

The prototypical muscarinic receptor antagonist is **atropine,** a naturally occurring alkaloid. Atropine is used clinically to induce mydriasis (pupil dilation) for ophthalmologic examinations, to reverse sinus bradycardia caused by excessive vagal tone in the setting of acute myocardial infarction or asystole, to inhibit excessive salivation and mucus secretion during surgery, to prevent vagal reflexes induced by surgical trauma of visceral organs, and to counteract the effects of muscarine poisoning from certain mushrooms. Because of its marginal activity at nicotinic receptors, extremely high doses of atropine are required for any effects to be seen at the NMJ. Similarly, because nicotinic receptors are primarily responsible for excitatory transmission at autonomic ganglia, atropine produces partial block at these sites only at relatively high doses.

**Scopolamine,** which differs from atropine by virtue of its significant CNS effects, is commonly used to prevent motion sickness. **Pirenzepine,** which is selective for $M_1$ and $M_4$ receptors, is an alternative to $H_2$-receptor antagonists in the treatment of peptic ulcers (see Chapter 44). **Ipratropium,** a synthetic quaternary ammonium compound, is more effective than β-adrenergic agonists in the treatment of chronic obstructive pulmonary disease, but less effective in treating asthma (see Chapter 45). In the past, anticholinergics were also used for treatment of Parkinson's disease; however, the development of levodopa has made this use obsolete.

### Nicotinic Receptor Antagonists

Selective nicotinic receptor antagonists are used primarily to produce **nondepolarizing (competitive) neuromuscular blockade** during surgical procedures. The many different nondepolarizing NMJ blocking agents all act by antagonizing nicotinic ACh receptors directly, thus preventing endogenous ACh binding and subsequent muscle cell depolarization. This leads to a flaccid paralysis similar in presentation to that of myasthenia gravis. The primary consideration used in selecting a specific agent is its duration of action, from the very long-lasting agents **(d-tubocurarine, pancuronium)** to those in the intermediate range **(vecuronium, rocuronium)** to the rapidly degraded compounds **(mivacurium)**. Because nicotinic receptors are found in autonomic ganglia as well as the NMJ, nondepolarizing blocking agents often have variable side effects associated with ganglionic blockade. These effects, as with the muscular paralysis, can be reversed by administration of AChE inhibitors.

Compounds with relatively selective antagonist activity at nAChRs are also used to induce autonomic blockade. The general effects of autonomic ganglionic blockade have been discussed previously and are listed in Table 7-2. Most commonly, **mecamylamine** and **trimethaphan** are administered when ganglionic blockade is desired. The only current use for these agents is in the treatment of hypertension in patients with acute dissecting aortic aneurysms, because the drugs lower blood pressure while simultaneously blunting the sympathetic reflexes that would normally cause a deleterious rise in pressure at the site of the tear.

## *Conclusion and Future Directions*

There are two major classes of cholinergic receptors—nicotinic and muscarinic. Nicotinic receptors are ligand-gated channels that require the direct binding of two acetylcholine molecules to open. These receptors comprise all of the cholinergic receptors at the neuromuscular junction ($N_M$), and they predominate at the autonomic ganglia ($N_N$). Thus, the primary cholinergic functions mediated by the nAChR include skeletal muscle contraction and autonomic activity. The predominant applications of pharmacologic agents directed at nAChRs are (1) neuromuscular blockade through competitive antagonists and depolarizing blockers, and (2) ganglionic blockade resulting in effector organ responses that are opposite to those produced by normal autonomic tone.

Muscarinic receptors are G protein-coupled receptors that bind acetylcholine and initiate signaling through a variety of intracellular pathways. These receptors are found at the autonomic ganglia and effector organs, where they mediate a parasympathetic response. The primary uses of muscarinic receptor agonists and antagonists are to modulate autonomic responses of effector organs. Both nicotinic and muscarinic receptors are ubiquitous in the CNS, where the effects of acetylcholine include analgesia, arousal, and attention. Because the relative roles of mAChRs and nAChRs in the brain and spinal cord are not fully understood, the most effective currently available CNS drugs increase endogenous cholinergic transmission by inhibiting the action of acetylcholinesterase, the enzyme that hydrolyzes ACh.

Although cholinergic pharmacology is a relatively mature field with a number of receptor-specific agents, the specificity of action of the various agents continues to be refined. The discovery of muscarinic receptor subtype diversity may lead to the development of agents specific for subtypes that are expressed in a tissue-specific pattern. Similarly, elucidation of the role of nicotinic receptor subunit diversity in the CNS may spur the development of more selective agents that modulate the activity of these receptor subtypes. Acetylcholinesterase inhibitors are among the few agents approved for the treatment of Alzheimer's disease. Although these agents do not decrease mortality, an increased understanding of the role played by acetylcholine in Alzheimer's disease could, in the future, lead to the development of more efficacious agents.

## *Suggested Readings*

Bowman WC. Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. Anesth Analg 1980;59:935–943. (*Discusses the basic physiology of receptors at the NMJ.*)

Changeux JP. Chemical signaling in the brain. Sci Am 1993;269: 58–62. (*Overview of the diversity of cholinergic receptors found in the CNS.*)

Sarter M. Cognitive functions of cortical acetylcholine: Toward a unifying hypothesis. Brain Res 1997;23:28–47. (*Discusses mechanisms by which acetylcholine modifies processing of cortical inputs.*)

Sarter M, Bruno JP, Turchi J. Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders. Ann NY Acad Sci 1999;877:378–382. (*More details about acetylcholine pathways in the CNS and their pathophysiology.*)

Urban MO. Central mechanisms in pain. Med Clin North Am 1999;83:585–597. (*Detailed review of the biochemical basis for CNS modulation of pain perception.*)

## Drug Summary Table Cholinergic Pharmacology

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF ACETYLCHOLINE SYNTHESIS**<br>*Mechanism—Inhibit intermediate synthetic steps (see Notes)* | | | | |
| **Hemicholinium-3** | None (experimental only) | | | Blocks transporter for choline uptake |
| **Vesamicol** | None (experimental only) | | | Blocks ACh-H$^+$ antiporter for transporting ACh into vesicles |
| **Botulinum toxin** | Achalasia, strabismus, blepharospasm, cosmetic | Overdose causes respiratory paralysis and death | | Prevents binding of cholinergic vesicles to cell membrane and subsequent release of ACh |
| **INHIBITORS OF ACETYLCHOLINE DEGRADATION**<br>*Mechanism—Inhibit acetylcholinesterase (AChE)* | | | | |
| **Edrophonium** | Diagnosis of myasthenia gravis, Eaton–Lambert, depolarizing blockade | Seizures | Pregnancy | Short-acting: 2–10 minutes; rapid onset of action makes it ideal for dx muscle weakness of unknown etiology |
| **Neostigmine**<br>**Pyridostigmine**<br>**Ambenonium** | Urinary/GI motility agent, glaucoma, NMJ diseases, reversal of anticholinergic toxicity, reversal of induced paralysis in surgery | | Do not administer in muscle weakness of unknown etiology, diabetes, asthma | These agents bond covalently to AChE, last 3–6 hours, polar. Neostigmine additionally has direct cholinergic agonist effect at $N_M$ receptors |
| **Physostigmine** | Reversal of anticholinergic drug poisoning (especially CNS toxicity), glaucoma, reversal of induced paralysis in surgery | Seizures | Do not administer in muscle weakness of unknown etiology, diabetes, asthma | Covalent bonding to AChE, lasts 3–6 hours, nonpolar structure makes it useful for combating CNS anticholinergic toxicity |
| **Diisopropyl fluorophosphate** | Anticholinergic toxicity (rarely used) | | | Organophosphate, lasts hundreds of hours, aging occurs, nonpolar. Pralidoxime rescues organophosphate poisoning |
| **Tacrine**<br>**Donepezil**<br>**Rivastigmine**<br>**Galantamine** | Delay symptoms of Alzheimer's disease | Hepatotoxicity, GI bleeding | | These agents do not improve mortality from Alzheimer's. Tacrine more strongly associated with hepatotoxicity |
| **MUSARINIC RECEPTOR AGONISTS**<br>*Mechanism—Stimulate muscarinic receptor activity* | | | | |
| **Methacholine** | Diagnosis of asthma | Vagomimetic activity | | High resistance to AChE, cardiac selective |
| **Carbachol** | Glaucoma, miotic agent | | Do not administer systemically | Also has significant nicotinic action |
| **Bethanechol** | Urinary tract motility agent for neurogenic bladder postop or postpartum | Heart block, syncope, cardiac arrest | | No nicotinic action |
| **Pilocarpine** | Miotic agent, treatment for xerostomia, Sjögren's | | Asthma, glaucoma | |

| | | | | |
|---|---|---|---|---|
| NICOTINIC RECEPTOR AGONISTS<br>*Mechanism—Stimulates nicotinic receptor channel opening* | | | | |
| **Succinylcholine** | Muscle relaxant for surgery or intubations | Malignant hyperthermia, hyperkalemia, rhabdomyolysis | Do not use in patients with burns, myopathy, NMJ diseases | Short-acting depolarizing blockade agent; causes transient fasciculations; short duration of action makes this drug of choice for paralysis during intubation |
| MUSCARINIC RECEPTOR ANTAGONISTS<br>*Mechanism—Selectively antagonize muscarinic receptors* | | | | |
| **Atropine** | Reversal of sinus bradycardia and heart block, counteract cholinergic toxicity, mydriatic agent for eye exams | | Glaucoma, tachycardia secondary to cardiac insufficiency or thyrotoxicosis | Mainly muscarinic activity, marginal nicotinic effect. Better at reversal of exogenous rather than endogenous cholinergic activity, minimal CNS activity, non-polar |
| **Scopolamine** | Motion sickness, antiemetic | | Glaucoma, tachycardia secondary to cardiac insufficiency or thyrotoxicosis | Has CNS activity |
| **Pirenzepine** | Gastric ulcers | | | Alternative agent to standard peptic ulcer disease therapies |
| **Ipratropium** | COPD, asthma | | Acute bronchospasm | Can treat Parkinson's disease but has been made obsolete by levodopa |
| NICOTINIC RECEPTOR ANTAGONISTS<br>*Mechanism—Selectively antagonize nicotinic receptors* | | | | |
| **Tubocurarine**<br>**Pancuronium** | Muscle relaxant for surgery | Anaphylaxis. Tubocurarine: arrhythmias, hypotension | Hypersensitivity to bromide (pancuronium only) | Long acting nondepolarizing neuromuscular blockade |
| **Vecuronium**<br>**Rocuronium** | Muscle relaxant for surgery | Anaphylaxis | Hypersensitivity to bromide (vecuronium only) | Intermediate acting nondepolarizing neuromuscular blockade, shorter duration of action results in better control of muscle paralysis for surgery |
| **Mivacurium** | Muscle relaxant for surgery | | | Short-acting nondepolarizing neuromuscular blockade |
| **Mecamylamine**<br>**Trimethaphan** | Hypertension in patients with acute dissecting aortic aneurysm | Unpredictable ganglionic blockade | | Ganglionic blockade makes these drugs desirable for aortic dissection, but inferior to other drugs for uncomplicated hypertension |

# 8

# Adrenergic Pharmacology

Freddie M. Williams and Timothy J. Turner

**Introduction**
**Case**
**Biochemistry and Physiology of Adrenergic Action**
  Catecholamine Synthesis, Storage, and Release
  Reuptake and Metabolism of Catecholamines
  Catecholamine Receptors
    *α-Adrenoceptors*
    *β-Adrenoceptors*
  Physiologic and Pharmacologic Effects of Endogenous Catecholamines
    *Epinephrine*
    *Norepinephrine*
    *Dopamine*
**Pharmacologic Classes and Agents**
  Inhibitors of Catecholamine Synthesis
  Inhibitors of Catecholamine Storage
    *Vesicular Transport*
    *Vesicular Storage*
    *Catecholamine Reuptake*
  Inhibitors of Catecholamine Metabolism
  Receptor Agonists
    *α-Adrenergic Agonists*
    *β-Adrenergic Agonists*
  Receptor Antagonists
    *α-Adrenergic Antagonists*
    *β-Adrenergic Antagonists*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Adrenergic pharmacology involves the study of agents that act on pathways mediated by the endogenous catecholamines norepinephrine, epinephrine, and dopamine. These neurotransmitters modulate the rate and force of cardiac contraction, the resistance (constriction and dilation) of blood vessels and bronchioles, the release of insulin, and the breakdown of fats. Drugs that target the synthesis, storage, release, and reuptake of norepinephrine and epinephrine, and that target the postsynaptic receptors for these transmitters, are frontline therapies for hypertension, depression, shock, asthma, angina, and many other disorders. This chapter examines the biochemical and physiologic basis for adrenergic action and then uses this biochemistry and physiology as a framework for discussing the action of a number of different classes of drugs. Because drugs acting on adrenergic pathways have multiple clinical applications, the concepts presented in this chapter are relevant throughout this book.

## ■ Case

The year is 1960. Phyllis Schweitz has felt depressed for a number of years. She has tried several different medications to alleviate her feelings of hopelessness and lack of motivation, but nothing seems to help. Recently, however, her doctor has prescribed iproniazid, a new medication purported to cure many cases of depression. He tells her that researchers think the drug acts by inhibiting an enzyme in her brain called MAO. (NB: This is one of the enzymes responsible for catecholamine degradation.) Because it is a new drug, its potential side effects are not well defined, so the doctor exhorts Phyllis to be careful to note and report any unusual effects of the medication.

Hopeful, but not expecting significant changes, Phyllis begins taking the medication. Within a few weeks, she begins to feel motivated and energetic for the first time in 20 years. Exuberant at her newly found sense of energy, Phyllis reclaims her past life as a debutante and socialite by hosting a gala wine and cheese reception. The best and brightest of the city are invited, and the party is looking to be a success. As she stands up to give thanks to her attendees, Phyllis celebrates with a large swig of her favorite 1954 Chianti. By the end of the party, Phyllis has a severe headache and nausea. Recalling her doctor's warning, Phyllis has a friend rush her to the nearest hospital. In the emergency room, the attending physician records a stunning 230/160 mm Hg blood pressure. Recognizing that Phyllis is experiencing a hypertensive emergency, the doctor quickly administers phentolamine (an α-adrenoceptor antagonist). Phyllis's blood pressure quickly normalizes, and the doctor's subsequent clinical investigation identifies a new, and now-famous, drug–food interaction involving monoamine oxidase (MAO) inhibitors.

## QUESTIONS:

1. What is the mechanistic explanation for the interaction of MAO inhibitors with red wine and aged cheese?
2. How does phentolamine act, and why was it useful in this clinical circumstance?
3. Why didn't the physician use a β-adrenoceptor antagonist to treat Phyllis' hypertension?
4. What drugs could have prevented the hypertensive crisis? (Your answer may include drugs not approved for clinical use.)

## BIOCHEMISTRY AND PHYSIOLOGY OF ADRENERGIC ACTION

The autonomic nervous system maintains homeostasis through the concerted action of its sympathetic and parasympathetic branches on a wide array of organ systems. The sympathetic nervous system prevails under conditions of stress, producing a "fight-or-flight" response that aids the organism in surviving such challenges. The following discussion presents the biochemistry of catecholamine action, from synthesis to metabolism to receptor activation. Then the physiologic roles of the endogenous catecholamines epinephrine, norepinephrine, and dopamine are discussed, with emphasis on the specificity of receptor expression among the major organs.

## CATECHOLAMINE SYNTHESIS, STORAGE, AND RELEASE

Catecholamines are synthesized by oxidation of the amino acid tyrosine. This synthesis occurs primarily at sympathetic nerve endings, but also to some degree in the neuronal cell bodies. Epinephrine synthesis predominates in adrenal medullary cells; whereas, most adrenergic neurons produce norepinephrine (Fig. 8-1). Tyrosine, the substrate for catecholamine synthesis, is transported into neurons via an aromatic amino acid transporter that uses the Na$^+$ gradient across the neuronal membrane to concentrate tyrosine, phenylalanine, tryptophan, and histidine. The first step in catecholamine synthesis, the oxidation of tyrosine to dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (TH), is the rate-limiting step, and, therefore, is an important regulatory step. DOPA is converted to dopamine by a generic aromatic amino acid decarboxylase.

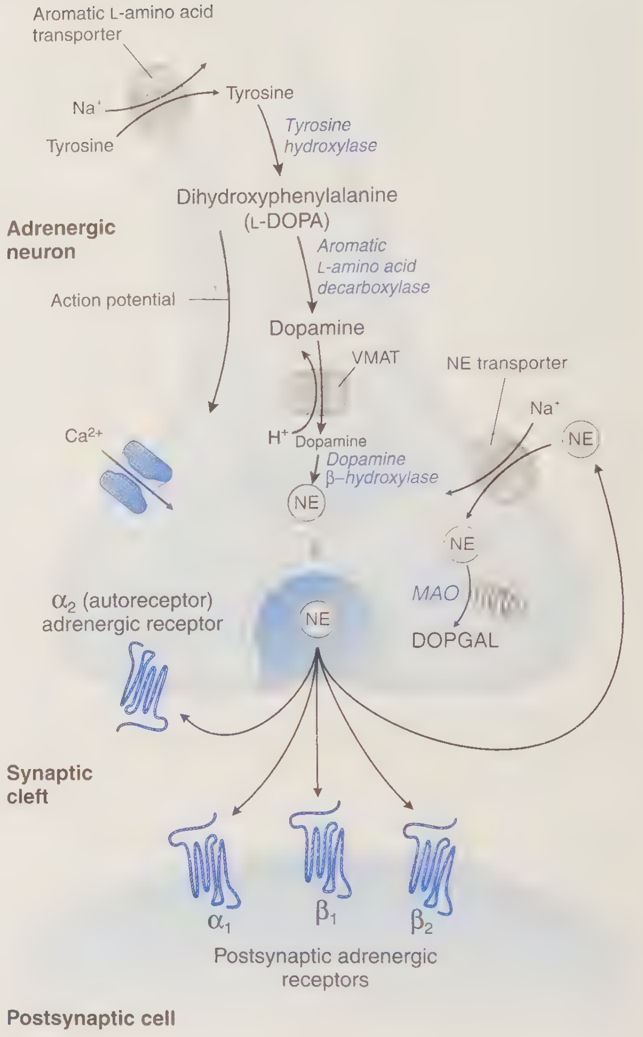


**Figure 8-1. Catecholamine Synthesis, Storage, Release, and Reuptake Pathways.** The endogenous catecholamines dopamine, norepinephrine and epinephrine are all synthesized from tyrosine. The rate-limiting step in catecholamine synthesis, the oxidation of cytoplasmic tyrosine to dihydroxyphenylalanine (L-DOPA), is catalyzed by the enzyme tyrosine hydroxylase. Aromatic L-amino acid decarboxylase then converts L-DOPA to dopamine. Vesicular monoamine transporter (VMAT) translocates dopamine (and other monoamines) into synaptic vesicles. In adrenergic neurons, intravesicular dopamine-β–hydroxylase converts dopamine to norepinephrine (NE). Norepinephrine is then stored in the vesicle until release. In adrenal medullary cells, norepinephrine returns to the cytosol, where phenylethanolamine N-methyltransferase (PNMT) converts norepinephrine to epinephrine. The epinephrine is then transported back into the vesicle for storage (not shown). α-Methyltyrosine inhibits tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis (not shown). Released norepinephrine can stimulate postsynaptic α$_1$-, β$_1$- or β$_2$-adrenergic receptors, or presynaptic α$_2$-adrenergic autoreceptors. Released norepinephrine can also be taken up into presynaptic terminals by the selective NE transporter. NE in the cytoplasm of the presynaptic neuron can be further taken up into synaptic vesicles by VMAT (not shown) or degraded to DOPGAL by mitochondrion-associated monoamine oxidase (MAO).

Dopamine is then hydroxylated at the 8-position (or β-position) by dopamine-β-hydroxylase to yield norepinephrine. In tissues that produce epinephrine, norepinephrine is then methylated on its amino group by phenylethanolamine-N-methyltransferase (PNMT).

The subcellular localization of these various synthetic steps is tied to the eventual storage of neurotransmitter. The conversion of tyrosine to dopamine occurs within the cytoplasm of the neuron. Dopamine is then transported into the synaptic vesicle by a 12-helix membrane-spanning proton antiporter called the **vesicular monoamine transporter (VMAT).** VMAT is also known as Uptake 2 (Fig. 8-2). This antiporter takes advantage of the proton gradient, generated by a proton ATPase in the vesicular membrane, to concentrate dopamine inside the vesicle. Dopamine inside the vesicle is converted to norepinephrine by dopamine-β-hydroxylase. Norepinephrine concentrations within the vesicle can reach 100 mM. To stabilize the osmotic pressure resulting from the very high concentration gradient for norepinephrine across the vesicle membrane, the norepinephrine is thought to condense with ATP. Consequently, ATP and norepinephrine are coreleased upon vesicle exocytosis.

In adrenal medullary cells, vesicular norepinephrine is transported or diffuses back into the cytoplasm, where PNMT converts norepinephrine to epinephrine. The epinephrine is then transported back into the vesicle for storage until its eventual release by exocytosis. It should be noted that VMAT is a relatively nonselective transporter capable of transporting many compounds structurally similar to norepinephrine; this property of VMAT has important pharmacologic consequences, as discussed below.

Catecholamine release is initiated by signals originating in an array of processing areas in the central nervous system (CNS), especially the limbic system. These CNS neurons project axons that synapse on sympathetic preganglionic neurons in the intermediolateral columns of the spinal cord. The preganglionic axons project to the sympathetic ganglia, where they release acetylcholine. This neurotransmitter initiates excitatory postsynaptic potentials in postganglionic neurons by activating nicotinic acetylcholine (ACh) receptors (cation-selective channels that depolarize the neuronal membrane). Such ganglionic blockers as **hexamethonium** and **mecamylamine** block the ganglionic nicotinic ACh receptor, without significant effects on the skeletal muscle isoform (see Chapter 7, Cholinergic Pharmacology). The sympathetic postganglionic axons form varicosities or *en passant* synapses in or on target organs. The arrival of an action potential at these endings opens voltage-gated $Ca^{2+}$ channels, and the ensuing $Ca^{2+}$ influx triggers exocytosis of the catecholamine-containing synaptic vesicles (with the notable exception that ACh is the transmitter used at sympathetic nerve endings in sweat glands). Norepinephrine rapidly diffuses away from the presynaptic varicosity and locally regulates tissue responses (e.g., smooth muscle tone) by activating postsynaptic adrenergic receptors. It has recently been recognized that adrenergic receptors are also located on presynaptic nerve endings; this may serve as an autoregulatory mechanism for modulating the extent of neurotransmitter release.

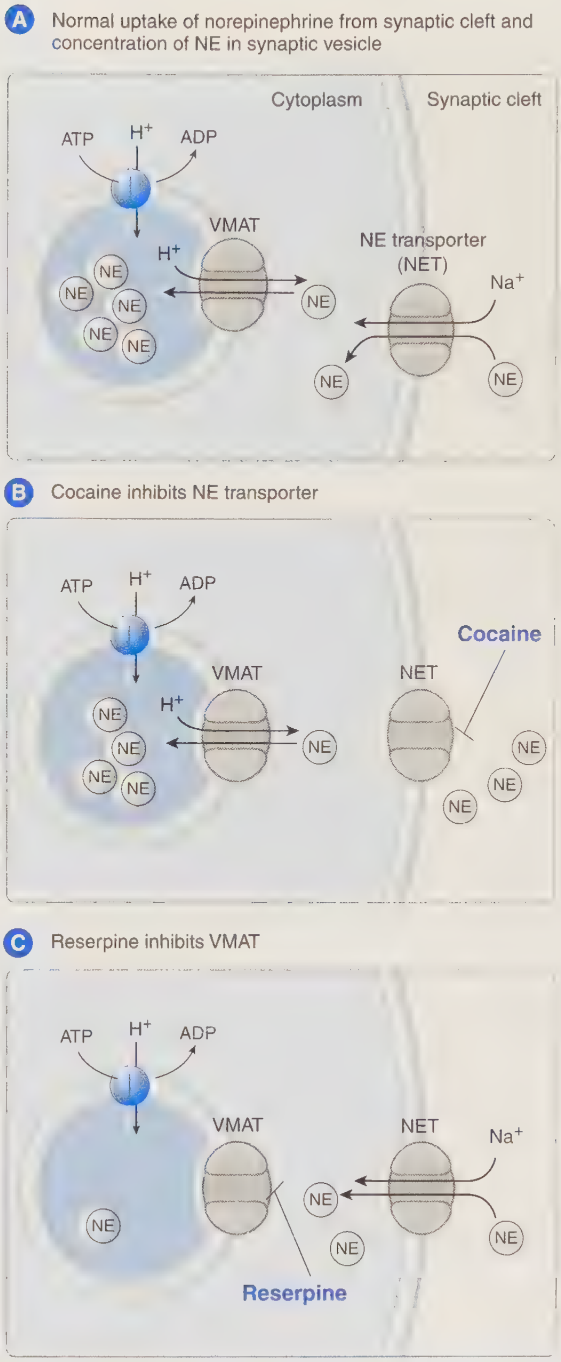

A
Normal uptake of norepinephrine from synaptic cleft and concentration of NE in synaptic vesicle
Cytoplasm
Synaptic cleft
ATP
H+
ADP
VMAT
H+
NE
NE transporter (NET)
Na+
B
Cocaine inhibits NE transporter
Cocaine
NET
C
Reserpine inhibits VMAT
Reserpine

**Figure 8-2. Mechanisms of Action of Cocaine and Reserpine.** **A.** Norepinephrine (NE) that has been released into the synaptic cleft can be taken up into the cytoplasm of the presynaptic neuron by the selective NE transporter (NET), a $Na^+$-NE co-transporter. Cytoplasmic NE is concentrated in synaptic vesicles by the nonselective vesicular monoamine transporter (VMAT), an $H^+$-monoamine antiporter. An $H^+$-ATPase uses the energy of ATP hydrolysis to concentrate protons in synaptic vesicles and, thereby, generate a transmembrane $H^+$ gradient. This $H^+$ gradient is used by VMAT to drive monoamine transport into the synaptic vesicle. **B.** Cocaine inhibits the NE transporter, allowing released NE to remain in the synaptic cleft for a longer period of time. By this mechanism, cocaine potentiates neurotransmission at adrenergic synapses. **C.** Reserpine inhibits the vesicular monoamine transporter, preventing the refilling of synaptic vesicles with NE and eventually depleting the adrenergic terminal of neurotransmitter. By this mechanism, reserpine inhibits neurotransmission at adrenergic synapses.

## REUPTAKE AND METABOLISM OF CATECHOLAMINES

Once a catecholamine molecule has exerted its effect at a postsynaptic receptor, termination of the response occurs by one of three mechanisms: (1) reuptake of catecholamine into the presynaptic neuron; (2) metabolism of catecholamine to an inactive metabolite; and (3) diffusion of the catecholamine away from the synaptic cleft. The first two of these mechanisms require specific transport proteins or enzymes, and therefore are targets for pharmacologic intervention.

Reuptake of catecholamine into the neuronal cytoplasm is mediated by a selective catecholamine transpoter (e.g., **norepinephrine transporter** or **NET**) that is also known as Uptake 1 (Fig. 8-2). This symporter uses the inward $Na^+$ concentration gradient to concentrate catecholamines in the cytoplasm of sympathetic nerve endings, thus limiting the postsynaptic response and allowing neurons to recycle the transmitter for reuse. Inside the nerve terminal, these catecholamines can be further concentrated in synaptic vesicles via VMAT, the same transporter used to transport dopamine into the vesicle during catecholamine synthesis. Thus, *the pool of catecholamines available for release comes from two sources: molecules that are synthesized de novo, and molecules that are recycled via neuronal reuptake.*

Catecholamine metabolism involves the two enzymes **monoamine oxidase (MAO)** and **catechol-O-methyltransferase (COMT)** (Fig. 8-3). MAO is a mitochondrial enzyme that is expressed in most neurons. It exists in two isoforms, MAO-A and MAO-B. The two isoforms have some ligand specificity: MAO-A preferentially degrades serotonin, norepinephrine, and dopamine, while MAO-B degrades dopamine more rapidly than serotonin and norepinephrine. COMT is a cytosolic enzyme that is expressed primarily in the liver.

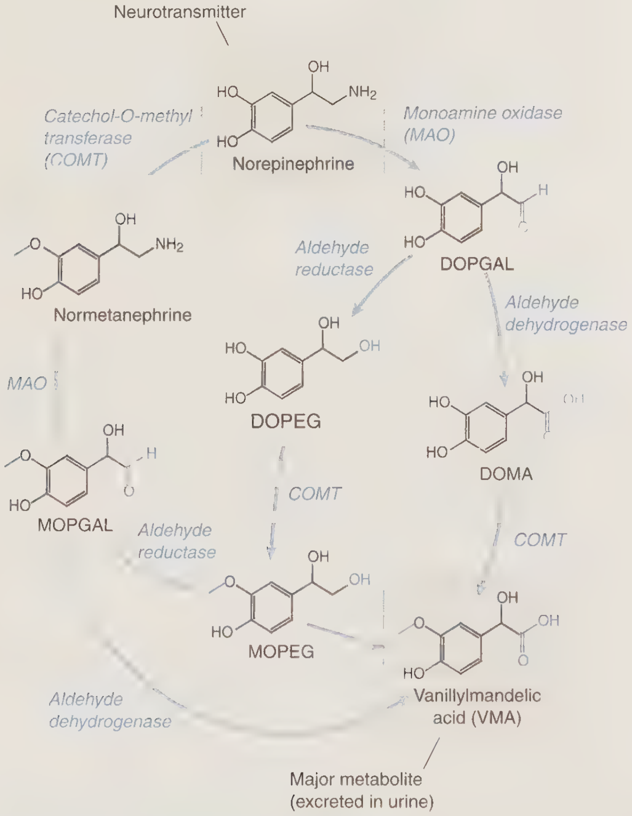


**Figure 8-3. Norepinephrine Metabolism.** Norepinephrine is degraded to metabolites by two main enzymes. Catechol-O-methyl transferase (COMT) is a widely distributed cytosolic enzyme; COMT in the liver is particularly important in the metabolism of circulating catecholamines. Monoamine oxidase (MAO), which is localized to the outer surface of mitochondria, is found in many monoaminergic (including adrenergic) neurons. COMT, MAO, aldehyde reductase, and aldehyde dehydrogenase metabolize catecholamines to multiple intermediates that are eventually excreted. Vanillylmandelic acid (VMA) is the major metabolite excreted in urine.

## CATECHOLAMINE RECEPTORS

Adrenergic receptors (also called adrenoceptors) are catecholamine receptors selective for norepinephrine and epinephrine. Very high (supraphysiologic) concentrations of dopamine can also activate some adrenoceptors. These receptors are divided into two main classes, termed $\alpha$ and $\beta$. All of the catecholamine receptor classes and subclasses are members of the G protein-coupled receptor superfamily (see Chapter 1, Drug–Receptor Interactions).

### $\alpha$-Adrenoceptors

$\alpha$-Adrenoceptors are divided into $\alpha_1$ and $\alpha_2$ subclasses (Table 8-1). The majority of $\alpha_1$-receptors signal via $G_q$-mediated pathways that generate $IP_3$, which mobilizes intracellular $Ca^{2+}$ stores, and DAG, which activates protein kinase C. $\alpha_1$-Receptors are expressed in vascular smooth muscle, genitourinary smooth muscle, intestinal smooth muscle, heart, and liver. In vascular smooth muscle cells, stimulation of $\alpha_1$-receptors causes increased intracellular [$Ca^{2+}$], activation of calmodulin, phosphorylation of myosin light chain, increased actin–myosin interaction, and muscle contraction (see Chapter 20, Pharmacology of Vascular Tone). The $\alpha_1$-receptor subtype is, therefore, important for mediating increases in blood pressure, and $\alpha_1$-receptor antagonists are logical therapies for hypertension. Because $\alpha_1$-receptors also cause contraction of genitourinary smooth muscle, $\alpha_1$-receptor antagonists are also used clinically in the symptomatic treatment of prostatic hypertrophy (see below).

$\alpha_2$-Adrenoceptors activate $G_i$, an inhibitory G protein. $G_i$ has multiple signaling effects, including inhibition of adenylyl cyclase (causing cAMP levels to fall), activation of G protein-coupled inward rectifier $K^+$ channels (causing membrane hyperpolarization), and inhibition of neuronal $Ca^{2+}$ channels. Each of these effects tends to decrease neurotransmitter release from the target neuron. $\alpha_2$-Receptors are found on both presynaptic neurons and postsynaptic cells. *Presynaptic $\alpha_2$-receptors function as autoreceptors to mediate feedback inhibition of sympathetic output.* $\alpha_2$-Receptors are also found on pancreatic $\beta$-cells and platelets, where they mediate inhibition of insulin release and inhibition of platelet aggregation, respectively. The latter observation has led to the development of antiplatelet agents that are specific inhibitors of platelet $\alpha_2$-receptors (see below). The main pharmacologic application of $\alpha_2$-receptors, however, has been in the treatment of hypertension. $\alpha_2$-Receptor agonists act at CNS sites to decrease sympathetic outflow to the periphery, resulting in decreased norepinephrine release at sympathetic nerve terminals and, therefore, decreased vascular smooth muscle contraction.

### $\beta$-Adrenoceptors

$\beta$-Adrenoceptors are divided into three subclasses, termed $\beta_1$, $\beta_2$, and $\beta_3$. All three subclasses activate a stimulatory G protein, $G_s$. $G_s$ activates adenylyl cyclase, leading to an increase

**TABLE 8-1** Adrenoceptor Actions

| RECEPTOR SUBTYPE | SIGNALING MEDIATORS | TISSUE | EFFECTS |
|---|---|---|---|
| $\alpha_1$ | $G_q/G_i/G_o$ | Vascular smooth muscle | Contraction |
| | | Genitourinary smooth muscle | Contraction |
| | | Intestinal smooth muscle | Relaxation |
| | | Heart | ↑ Inotropy and excitability |
| | | Liver | Glycogenolysis and gluconeogenesis |
| $\alpha_2$ | $G_i/G_o$ | Pancreatic β-cells | ↓ Insulin secretion |
| | | Platelets | Aggregation |
| | | Nerve | ↓ Norepinephrine release |
| | | Vascular smooth muscle | Contraction |
| $\beta_1$ | $G_s$ | Heart | ↑ Chronotropy and inotropy |
| | | Heart | ↑ AV-node conduction velocity |
| | | Renal juxtaglomerular cells | ↑ Renin secretion |
| $\beta_2$ | $G_s$ | Smooth muscle | Relaxation |
| | | Liver | Glycogenolysis and gluconeogenesis |
| | | Skeletal muscle | Glycogenolysis and $K^+$ uptake |
| $\beta_3$ | $G_s$ | Adipose | Lipolysis |

in the level of intracellular cAMP. Increased cAMP activates protein kinases (especially protein kinase A), which phosphorylate cellular proteins, including ion channels. Because few signaling differences exist among the different β-adrenoceptor subtypes, their pharmacologic selectivity seems to reside in the tissue-specific distribution of each receptor subtype.

$\beta_1$-Adrenoceptors are localized primarily to the heart and kidney. In the kidney, these receptors are present mainly on renal juxtaglomerular cells, where receptor activation causes renin release (see Chapter 19, Pharmacology of Volume Regulation). Stimulation of cardiac $\beta_1$-receptors causes an increase in both inotropy (force of contraction) and chronotropy (heart rate). The inotropic effect is mediated by increased phosphorylation of $Ca^{2+}$ channels, including calcium channels in the sarcolemma and phospholamban in the sarcoplasmic reticulum (see Chapter 18, Pharmacology of Cardiac Contractility). The increased chronotropy results from a $\beta_1$-mediated increase in the rate of phase 4 depolarization of sinoatrial node pacemaker cells. Both effects contribute to increased cardiac output (recall that cardiac output = heart rate × stroke volume). Activation of $\beta_1$-receptors also increases conduction velocity in the atrioventricular (AV) node because the increased $Ca^{2+}$ entry promoted by $\beta_1$-receptors increases the rate of depolarization of AV node cells.

The important effects of $\beta_1$-adrenoceptors on the force of contraction and heart rate make antagonists of this receptor subtype attractive agents in the treatment of hypertension and angina. $\beta_1$-Adrenoceptor antagonists are also used to prevent a second myocardial infarction in patients who have already sustained one such event and, somewhat counterintuitively, in the treatment of mild-to-moderate congestive heart failure. Because $\beta_1$-adrenoceptor antagonists slow AV-nodal conduction velocity, these agents are used to treat some forms of supraventricular tachycardia (see Chapter 17, Pharmacology of Cardiac Rhythm).

$\beta_2$-Adrenoceptors are expressed in smooth muscle, liver, and skeletal muscle. In smooth muscle, receptor activation stimulates $G_s$, adenylyl cyclase, cAMP, and protein kinase A. Protein kinase A phosphorylates several contractile proteins, leading to relaxation of the contractile apparatus. Evidence also suggests that $\beta_2$-adrenoceptor activation may relax bronchial smooth muscle by $G_s$-independent activation of $K^+$ channels. Increased $K^+$ ion flux leads to bronchial smooth muscle cell hyperpolarization, and therefore, opposes the depolarization necessary to elicit contraction. The powerful relaxation of bronchial smooth muscle mediated by $\beta_2$-receptors makes inhaled $\beta_2$-agonists useful agents in the treatment of asthma. In hepatocytes, activation of the $G_s$ signaling cascade initiates a series of intracellular phosphorylation events that culminate in glycogen phosphorylase activation and glycogen catabolism. The end result of $\beta_2$-adrenoceptor stimulation of hepatocytes is, therefore, an increase in plasma glucose. In skeletal muscle, activation of these same signaling pathways stimulates glycogenolysis and promotes $K^+$ uptake.

It has recently been discovered that $\beta_3$-adrenoceptors are expressed specifically in adipose tissue. Stimulation of $\beta_3$-receptors leads to an increase in lipolysis. This physiologic action has led to speculation that $\beta_3$-agonists may be useful in the treatment of obesity and noninsulin-dependent diabetes mellitus, but such pharmacologic agents remain to be developed for clinical use.

## PHYSIOLOGIC AND PHARMACOLOGIC EFFECTS OF ENDOGENOUS CATECHOLAMINES

The endogenous catecholamines epinephrine and norepinephrine act as agonists at both α- and β-adrenoceptors. At supraphysiologic concentrations, dopamine can also act as an agonist at α- and β-receptors. The overall effect of each neurotransmitter is complex and depends on the concentration of the agent and on whether the α- or β-effect dominates in the tissue under consideration.

### Epinephrine

Epinephrine stimulates both α- and β-adrenoceptors. *At low concentrations, epinephrine has predominantly $\beta_1$ and $\beta_2$ effects, while at high concentrations, its $\alpha_1$-effects predominate.* Acting at $\beta_1$-receptors, epinephrine increases cardiac contractile force and cardiac output, with consequent increases in cardiac oxygen consumption and systolic blood pressure. Vasodilation mediated by $\beta_2$-receptors causes a decrease in peripheral resistance and a consequent decrease in diastolic blood pressure. Stimulation of $\beta_2$-receptors also increases blood flow to skeletal muscle, relaxes bronchial smooth muscle, and increases the concentrations of glucose and free fatty acids in the blood. Note that these $\beta_1$- and $\beta_2$-effects are all components of the "fight-or-flight" response. Epinephrine is used to treat acute asthma and anaphylaxis; high doses of topical epinephrine cause vasoconstriction and, thereby, prolong the action of local anesthetics. Epinephrine has a rapid onset and a brief duration of action, and is ineffective orally. The increased cardiac excitability induced by epinephrine can lead to cardiac arrhythmias, and the sharp rise in blood pressure can provoke cerebral hemorrhage.

### Norepinephrine

*Norepinephrine has actions at $\alpha_1$- and $\beta_1$-receptors, but relatively little action at $\beta_2$-receptors.* Because of the lack of action at $\beta_2$-receptors, systemic administration of norepinephrine increases not only systolic blood pressure ($\beta_1$-effect) but also diastolic blood pressure and total peripheral resistance. Norepinephrine also increases heart rate, but this effect is usually overcome by reflex vagal activity in response to increased blood pressure. Therefore, norepinephrine increases stroke volume, but cardiac output remains unchanged because heart rate is ultimately decreased. Although norepinephrine has limited therapeutic value, this neurotransmitter is sometimes used in the treatment of low blood pressure (shock).

### Dopamine

Although dopamine is a prominent CNS neurotransmitter, systemic administration of this compound has few CNS effects because it does not readily cross the blood–brain barrier. Dopamine activates one or more subtypes of catecholamine receptor in peripheral tissues, and the predominant effect is dependent on the local concentration of the compound. At low doses (< 2 μg/kg per min), a continuous intravenous infusion of dopamine acts predominately on D1 dopaminergic receptors in renal, mesenteric, and coronary vascular beds (hence the term "renal dose" of dopamine). Stimulation of D1 dopaminergic receptors activates adenylyl cyclase in vascular smooth muscle cells, leading to increased cAMP levels and, ultimately, to vasodilation. At higher (supraphysiological) dosing rates (2–10 μg/kg per min), dopamine acts as a positive inotrope through its activation of $\beta_1$-adrenergic receptors. At still higher rates (> 10 μg/kg per min), dopamine acts on vascular $\alpha_1$-adrenergic receptors to cause vasoconstriction. Dopamine is used in the treatment of shock, particularly in states of shock caused by low cardiac output and accompanied by compromised renal function leading to oliguria.

## PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic intervention is possible at each of the major steps in catecholamine synthesis, storage, release, metabolism, and receptor activation. The following discussion presents the various classes of agents in the order of their action on adrenergic pathways, from neurotransmitter synthesis to receptor activation. Because of their clinical importance, the balance of the discussion addresses receptor agonists and antagonists.

## INHIBITORS OF CATECHOLAMINE SYNTHESIS

Inhibitors of catecholamine synthesis have limited clinical utility because such agents nonspecifically inhibit the formation of all catecholamines (see Fig. 8-1). **α-Methyltyrosine** is a structural analogue of tyrosine that is transported into nerve terminals, where it inhibits tyrosine hydroxylase, the first enzyme in the catecholamine biosynthesis pathway. This agent is used occasionally in the treatment of hypertension associated with pheochromocytoma (a tumor, involving cells of the adrenal medulla, that produces norepinephrine and epinephrine).

## INHIBITORS OF CATECHOLAMINE STORAGE

Drugs in this mechanistic class affect one of three steps: vesicular transport, vesicular storage, or catecholamine reuptake. The effect of these drugs can be complex. Recall that catecholamines originate from two pools—de novo synthesis and recycled transmitter. An agent that inhibits catecholamine transport into vesicles or displaces catecholamine from vesicles can have two effects. In the short term, the agent can increase the net release of catecholamine from the synaptic terminal, and thus increase sympathetic stimulation. Over a longer time period, however, the agent depletes the pool of available catecholamine and, thus, acts as a **sympatholytic** (inhibitor of sympathetic activity) (Fig. 8-4).

### Vesicular Transport

**Reserpine** binds tightly to the vesicular antiporter VMAT (see Figs. 8-1 and 8-2). The drug irreversibly damages VMAT, resulting in vesicles that lose their ability to concentrate and store norepinephrine and dopamine. At low doses, reserpine causes neurotransmitter leak into the cytoplasm, where the catecholamine is destroyed by intraneuronal MAO. At high doses, the rate of transmitter leak can be sufficiently high to overwhelm the MAO that is present in the presynaptic neuron. Under these conditions, there is a very high concentration of transmitter in the neuronal cytoplasm, and transmitter can exit from the cytoplasm to the synaptic space through NET acting in reverse. The massive efflux of catecholamine has a transient sympathomimetic effect. Because reserpine's inhibition of VMAT is irreversible, new storage vesicles

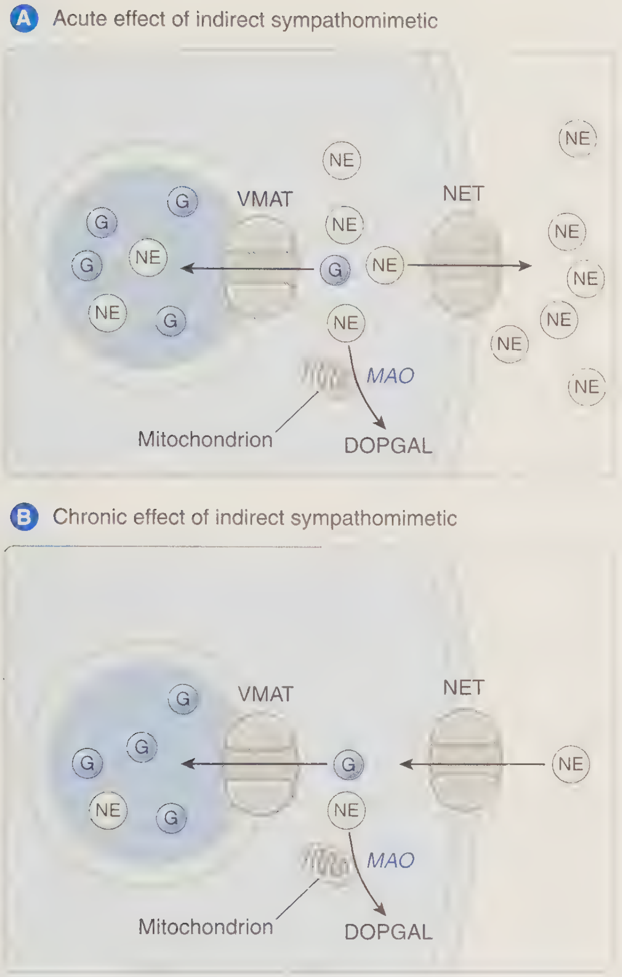

A
Acute effect of indirect sympathomimetic
G
VMAT
NE
NET
MAO
Mitochondrion
DOPGAL
B
Chronic effect of indirect sympathomimetic
G
VMAT
NE
NET
MAO
Mitochondrion
DOPGAL

**Figure 8-4. Acute and Chronic Effects of Indirect Sympathomimetics.** Indirect sympathomimetics have different effects on sympathetic outflow depending on whether they are administered acutely or chronically. **A.** Administered acutely, an indirect sympathomimetic such as guanethidine (G) displaces norepinephrine (NE) that is stored in the synaptic vesicles of adrenergic neurons. This results in a massive efflux of norepinephrine through the NE transporter acting in reverse; the resultant flooding of the synapse with norepinephrine causes marked sympathetic stimulation. **B.** Administered chronically, an indirect sympathomimetic such as guanethidine (G) is concentrated in synaptic vesicles and replaces norepinephrine. In addition, monoamine oxidase (MAO) degrades the small pool of norepinephrine that remains in the cytoplasm. Both of these effects contribute to decreased sympathetic stimulation.

must be synthesized and transported to the nerve terminal to restore proper vesicular function. This recovery phase may last for days to weeks after an individual stops taking reserpine. Reserpine can also be used experimentally to assess whether drugs need to be concentrated in presynaptic terminals to exert their actions. In the past, reserpine was used to treat hypertension. However, the irreversible nature of its action, and its association with psychotic depression, make it an unattractive agent now that safer, more efficacious drugs have been discovered for the treatment of hypertension.

## Vesicular Storage

Drugs affecting vesicular storage have several different mechanisms of action. All such drugs displace norepinephrine from synaptic vesicles, and some, acting as agonists, directly stimulate adrenoceptors as well. For this reason, agents in this class are typically called **indirect sympathomimetics** if they have no direct effect on adrenoceptors, and **mixed sympathomimetics** if they have both direct and indirect actions. Indirect sympathomimetics must be transported both into the nerve terminal and into synaptic vesicles to exert their pharmacologic effect. For this reason, inhibitors of NET (see below) decrease or negate the effect of indirect sympathomimetics.

**Tyramine** is the prototype indirect sympathomimetic. This compound is a dietary amine that is ordinarily metabolized in the gastrointestinal tract and liver by MAO. In patients taking MAO inhibitors (MAOIs, see below), tyramine is absorbed in the gut, transported through the bloodstream, and taken up by sympathetic neurons, where it is transported into synaptic vesicles by VMAT. By this mechanism, an acute challenge with large amounts of tyramine can cause acute displacement of vesicular norepinephrine and massive nonvesicular release of norepinephrine from the nerve terminal via reversal of NET. Fermented foods such as red wine and aged cheese possess high concentrations of dietary tyramine; this is why, in the introductory case, Phyllis developed a hypertensive crisis shortly after her wine and cheese party. Although tyramine itself is poorly retained in synaptic vesicles, its hydroxylated metabolite **octopamine** (whose synthesis is catalyzed by vesicular dopamine β-hydroxylase) can be stored at high concentrations in the vesicles. Under conditions of chronic MAOI treatment and modest dietary tyramine intake, norepinephrine may gradually be replaced by octopamine in these vesicles. Because **octopamine** has little agonist activity at mammalian adrenoceptors, postsynaptic responses to sympathetic stimulation may gradually be diminished, leading to the side effect of postural hypotension (see below).

**Guanethidine** is also an indirect sympathomimetic. This agent (like tyramine) is actively transported into neurons by NET, where it concentrates in transmitter vesicles and displaces norepinephrine, leading to gradual depletion of norepinephrine (see Fig. 8-4). Guanethidine (like octopamine) does not stimulate postsynaptic adrenoceptors, so its vesicular release upon sympathetic stimulation causes no postsynaptic response. Guanethidine and octopamine, therefore, represent a class of compounds known as "false neurotransmitters." In the past, guanethidine was used to treat uncontrolled hypertension. Guanethidine inhibits cardiac sympathetic nerves, leading to reduced cardiac output, and it blocks sympathetic-mediated vasoconstriction, leading to reduced cardiac preload. Symptomatic hypotension following exercise or standing up ("postural hypotension") may result from guanethidine's inhibition of these sympathetic responses. **Guanadrel** is another agent that acts as a false neurotransmitter. As with guanethidine, this drug can be used in the treatment of hypertension, but it is no longer a first-line agent. The side effect profile of guanadrel is similar to that of guanethidine.

**Amphetamine** is the prototype mixed sympathomimetic. This agent (1) displaces endogenous catecholamines (similar to tyramine), (2) inhibits MAO, and (3) acts as an agonist at peripheral α-adrenoceptors and β-adrenoceptors. It is also a potent adrenoceptor agonist in the CNS (the N-methyl derivative **methamphetamine** is even more so) and has marked behavioral effects including increased alertness, decreased fatigue, depressed appetite, and insomnia. Thus, amphetamine is used to treat depression and narcolepsy (recurrent attacks of drowsiness and sleep during the daytime) and to suppress appetite. Its side effects can be substantial, including fatigue and depression following the period of central stimulation. **Ephedrine, pseudoephedrine,** and **phenylpropanolamine** are mixed-acting sympathomimetics with actions less marked than those of amphetamine. These drugs are widely used as over-the-counter decongestants and are often found in cold remedies and appetite suppressants. **Methylphenidate,** a structural analogue, is widely used in psychiatry to treat attention-deficit hyperactivity disorder (ADHD) in children; its major effect is thought to be related to enhanced attention. For all these agents, psychologic and physiologic dependence and tolerance can occur. The mixed sympathomimetics increase both systolic and diastolic blood pressure, and individuals taking these drugs may experience restlessness, dizziness, tremor, irritability, confusion, aggressiveness, erectile dysfunction, anxiety, paranoid hallucinations, panic, and suicidal or homicidal ideations. The severity of the side effect profile is related to the efficacy of the individual agent as a CNS stimulant. Methamphetamine HCl (“crank” or “crystal meth”) is an important drug of abuse, especially in the western United States, where supplies are cheap and plentiful.

### Catecholamine Reuptake

Inhibitors of catecholamine reuptake can exert an acute and powerful sympathomimetic effect by prolonging the amount of time that released neurotransmitter remains in the synaptic cleft. **Cocaine** is a potent inhibitor of NET; unlike other uptake inhibitors (such as imipramine and fluoxetine), cocaine nearly eliminates catecholamine transport (see Fig. 8-2). Cocaine is a controlled substance with high abuse potential. It is used occasionally as a local anesthetic (see Chapter 9, Local Anesthetic Pharmacology), but its most important role is as an agent of abuse (see Chapter 16, Pharmacology of Chemical Dependence and Addiction).

The **tricyclic antidepressants (TCAs)**, such as **imipramine** and **amitriptyline,** inhibit NET-mediated reuptake of norepinephrine into presynaptic terminals and, thus, allow accumulation of norepinephrine in the synaptic cleft. The TCAs are promiscuous, however, inhibiting serotonin as well as norepinephrine reuptake into presynaptic terminals and blocking serotonergic, α-adrenergic, histaminergic, and muscarinic receptors at therapeutic doses. Not surprisingly, therefore, the TCAs display a prominent side effect profile. One hallmark of TCA therapy for depression is that, although these drugs begin inhibiting norepinephrine and serotonin reuptake immediately, there is a latency period of several weeks before improvement in symptoms is seen. The molecular mechanism responsible for this delayed onset of action remains the subject of investigation. Paradoxically, the tricyclics may cause both postural hypotension (through α-adrenergic blockade) and sinus tachycardia (through potentiation of norepinephrine action in cardiac sympathetic nerves). At high doses or in overdose, TCAs can have a quinidine-like effect on cardiac ion channels and, thereby, induce arrhythmia (see Chapter 17). Because of their important role in the treatment of depression, TCAs are discussed in more detail in Chapter 12, Pharmacology of Affective Disorders.

## INHIBITORS OF CATECHOLAMINE METABOLISM

**Monoamine oxidase inhibitors (MAOIs)** prevent secondary deamination of catecholamines following reuptake of the catecholamines into presynaptic terminals. Therefore, more catecholamines can accumulate in presynaptic stores (vesicles) and be released during each action potential. Most MAOIs are oxidized by MAO to reactive intermediates, which then act as irreversible inhibitors of MAO. Nonselective agents in this class (i.e., agents that inhibit both MAO-A and MAO-B) include **phenelzine, iproniazid** (the drug used in the introductory case), and **tranylcypromine.** Selective inhibitors of MAO-A or MAO-B include **clorgyline,** which is selective for MAO-A and **selegiline,** which is selective for MAO-B. **Brofaromine, befloxatone,** and **moclobemide** are newer, reversible inhibitors of MAO-A.

As with the tricyclic antidepressants, MAOIs are mainly used to treat depression. Selegiline is approved for the treatment of Parkinson’s disease; its mechanism of action may include both potentiation of dopamine in the remaining nigrostriatal neurons and decreased formation of neurotoxic intermediates. As noted above, patients taking MAOIs should avoid eating certain fermented foods containing large amounts of tyramine and other monoamines, because MAOIs block oxidative deamination of these monoamines in the gastrointestinal tract and liver, allowing them to enter the circulation and precipitate a hypertensive crisis. Concomitant use of MAOIs and selective serotonin reuptake inhibitors (SSRIs) is also contraindicated because this can precipitate “**serotonin syndrome,**” characterized by restlessness, tremors, seizures, and possibly coma and death. The reversible inhibitors of MAO-A may be less prone to such effects and interactions. MAOIs and SSRIs are also discussed in Chapters 11 and 12.

## RECEPTOR AGONISTS

Many adrenergic drugs act directly on adrenoceptors. These “direct sympathomimetics” alter physiologic function by activating one or more subtypes of adrenoceptor. Because of the important role that adrenoceptors play in mediating vascular tone, smooth muscle tone, and cardiac contractility, selective agonists and antagonists of these various receptors are mainstays of therapy for hypertension, asthma, and myocardial infarction. In the following discussion, the agents are organized according to receptor subtype specificity (see Table 8-1 for an overview of the relevant receptor subtypes).

### α-Adrenergic Agonists

As noted previously, both epinephrine and norepinephrine nonspecifically activate α-adrenoceptors, making these natural compounds (especially norepinephrine) useful in the treatment of shock. A number of drugs have been developed that stimulate α-receptors more selectively. In fact, all of the clinically available pharmacologic agents have some degree of selectivity for either $\alpha_1$- or $\alpha_2$-receptors. This selectivity is likely to increase the utility of the drugs, because $\alpha_1$- and $\alpha_2$-adrenoceptors often mediate opposing physiologic effects.

The $\alpha_1$-selective adrenergic agonists increase peripheral vascular resistance and thereby maintain or elevate blood pressure. These drugs may also cause sinus bradycardia through activation of reflex vagal responses. Systemically administered $\alpha_1$-agonists, such as **methoxamine,** have limited clinical use, but are sometimes employed in the treatment of hypotension or shock. A number of topically administered $\alpha_1$-agonists, such as **phenylephrine, oxymetazoline,** and **tetrahydrazoline,** are used in the nonprescription remedies *Afrin* and *Visine* (and others) to constrict vascular smooth muscle in the symptomatic relief of nasal congestion and ophthalmic hyperemia. Unfortunately, rebound hypersensitivity and return of symptoms often accompany use of these remedies. Phenylephrine is also used intravenously in the treatment of shock.

The $\alpha_2$-selective agonists activate $\alpha_2$-receptors in the cardiovascular control centers of the CNS and, thus, inhibit sympathetic nervous system stimulation of the heart and vasculature. These agents may also inhibit the release of norepinephrine from peripheral nerve endings through the activation of presynaptic $\alpha_2$-receptors. Although both of these effects can result in lowering blood pressure, the central nervous system effect seems to be more important. On occasion, these agents may cause an acute rise in blood pressure because of their action at postsynaptic $\alpha_2$-receptors in vascular smooth muscle, followed by a more prolonged decrease in blood pressure because of stimulation of presynaptic $\alpha_2$-receptors.

**Clonidine** is the best characterized $\alpha_2$-agonist. This drug is prescribed commonly for treatment of hypertension; it is also used as a sympatholytic to treat symptoms associated with drug withdrawal. Adverse side effects include bradycardia caused by decreased sympathetic activity and increased vagal activity, as well as dry mouth and sedation. Other centrally acting $\alpha_2$-agonists include the seldom-used agents **guanabenz** and **guanfacine.** These agents have side effect profiles similar to that of clonidine. Among these three agents, guanabenz is the most selective for $\alpha_2$-adrenoceptors; this agent may therefore have fewer side effects than clonidine and guanfacine.

**Methyldopa** is a precursor (pro-drug) to the $\alpha_2$-agonist methylnorepinephrine. Endogenous enzymes catalyze the metabolism of methyldopa to methylnorepinephrine, and the methylnorepinephrine is then released by the adrenergic nerve terminal, where it can act presynaptically as an $\alpha_2$-agonist. This action results in decreased sympathetic outflow from the CNS, and, thus, a lowering of blood pressure in hypertensive patients. Because methyldopa use can be associated with rare hepatotoxicity and autoimmune hemolytic anemia, this drug is not a first-line agent in the treatment of hypertension. Because it has proved safe in pregnancy, however, it is often the drug of choice for the treatment of hypertension during pregnancy.

## β-Adrenergic Agonists

Stimulation of $\beta_1$-adrenergic receptors causes an increase in heart rate and the force of cardiac contraction, resulting in increased cardiac output, while stimulation of $\beta_2$-adrenergic receptors causes relaxation of vascular, bronchial, and gastrointestinal smooth muscle. **Isoproterenol** is a nonselective β-agonist that can be used to relieve bronchoconstriction. This drug lowers peripheral vascular resistance and diastolic blood pressure (a $\beta_2$ effect), while systolic blood pressure remains the same or is increased (a $\beta_1$ effect). Because isoproterenol is a positive inotrope (increases cardiac contractility) and chronotrope (increases heart rate), cardiac output is increased. Isoproterenol causes less hyperglycemia than does epinephrine, because the former agent stimulates β-adrenergic activation of insulin secretion. Because isoproterenol is a nonselective activator of $\beta_1$- and $\beta_2$-adrenoceptors, and its use for relief of bronchoconstriction in asthma is often accompanied by unwanted cardiac side effects, this drug has in recent years been widely supplanted by newer $\beta_2$-selective agonists (see below).

**Dobutamine** has classically been described as a $\beta_1$-selective agonist. It is now appreciated, however, that the overall effect of dobutamine depends on the differential effects of the two stereoisomers contained in the racemic mixture (see Chapter 1, Drug–Receptor Interactions, for a discussion of stereoisomers). The (−) isomer acts as both an $\alpha_1$-agonist and a weak $\beta_1$-agonist, whereas, the (+) isomer acts as both an $\alpha_1$-antagonist and a potent $\beta_1$-agonist. The $\alpha_1$-agonist and antagonist properties effectively cancel each other out when the racemic mixture is administered, and the observed clinical result is that of a selective $\beta_1$-agonist. This agent has more prominent inotropic than chronotropic effects, resulting in increased contractility and cardiac output. Dobutamine is used clinically in the acute management of decompensated heart failure.

$\beta_2$-selective agonists are valuable in the treatment of asthma, because stimulation of $\beta_1$-adrenoceptors in the heart by nonselective β-agonists causes uncomfortable (and occasionally dangerous) cardiac side effects. Drug delivery devices have further facilitated selective stimulation of $\beta_2$-adrenoceptors in the target tissue of interest. For example, the use of aerosolizing inhalers allows dosing at the distal airways, where the drug is most needed. Pulmonary delivery also lowers the amount of drug that is delivered systemically, and drugs delivered in this way cause less activation of cardiac $\beta_1$-receptors and skeletal muscle $\beta_2$-receptors. The most important effects of these agents are relaxation of bronchial smooth muscle and decrease in airway resistance. $\beta_2$-Selective agonists are not completely specific for $\beta_2$-receptors, however, and adverse effects can include skeletal muscle tremor (through $\beta_2$-stimulation) and tachycardia (through $\beta_1$-stimulation).

**Metaproterenol** is the prototype $\beta_2$-selective agonist. This drug is used to treat obstructive airway disease and acute bronchospasm. **Terbutaline** and **albuterol** are two other agents in this class that have similar efficacy and duration of action. **Salmeterol** is a long-acting $\beta_2$-agonist—its effects last for about 12 hours. The long duration of action makes salmeterol ineffective for immediate relief of bronchoconstriction, but this drug may provide long-term prophylaxis against bronchoconstriction. The clinical utility of $\beta_2$-selective agonists is discussed more fully in Chapter 45, Integrative Inflammation Pharmacology: Asthma.

## RECEPTOR ANTAGONISTS

Antagonists at α- and β-adrenoceptors ("sympatholytics") are among the most widely used medications in clinical practice, because of the wide range of disease states that respond to modulation of adrenoceptor activity. Accordingly, much research has focused on the development of specific blockers of the α- and β-adrenoceptor subtypes.

## α-Adrenergic Antagonists

α-Adrenergic antagonists block endogenous catecholamines from binding to $\alpha_1$- and $\alpha_2$-adrenoceptors. By inhibiting vasoconstriction, these agents cause vasodilation, decreased blood pressure, and decreased peripheral resistance. The baroreceptor reflex usually attempts to compensate for this fall in blood pressure, resulting in reflex increases in heart rate and cardiac output. **Phenoxybenzamine** blocks both $\alpha_1$- and $\alpha_2$-receptors irreversibly. A progressive decrease in peripheral resistance results from the block of $\alpha_1$-receptors. This, in turn, leads to an increase in cardiac output through reflex sympathetic nerve stimulation. Blockade of $\alpha_2$-autoreceptors allows more norepinephrine to be released by noradrenergic neurons, and tachycardia ensues from the resulting increase in $\beta_1$-receptor stimulation. In addition, phenoxybenzamine inhibits catecholamine uptake into both adrenergic nerve terminals and extraneuronal tissues. Because of its many direct and indirect effects on the sympathetic nervous system, phenoxybenzamine is generally employed only in the preoperative management of pheochromocytoma, although it is used occasionally in the treatment of benign prostatic obstruction. **Phentolamine** is a reversible, nonselective α-adrenoceptor antagonist. This drug can also be used in the preoperative management of pheochromocytoma. Phentolamine was the ideal agent for use in introductory case, because it blocked the α-adrenergic–mediated vasoconstriction that caused Phyllis's hypertension.

**Prazosin** has a 1,000-fold greater affinity for $\alpha_1$-receptors than for $\alpha_2$-receptors. Its selective blockade of $\alpha_1$-receptors in arterioles and veins results in decreased peripheral vascular resistance and decreased venous return to the heart. Because of this reduction in cardiac preload, prazosin has little tendency to increase cardiac output and heart rate. Thus, reflex tachycardia does not usually occur. Cardiovascular applications of prazosin include the treatment of primary systemic hypertension and congestive heart failure. Because patients may experience marked postural hypotension and syncope with the first dose, this dose is generally prescribed in small quantities at bedtime (to ensure that the patient remains supine). Other agents in this class include **terazosin** and **doxazosin;** these agents have a longer half-life than prazosin, allowing less frequent dosing.

Because $\alpha_1$-adrenoceptors mediate contraction of genitourinary as well as vascular smooth muscle, $\alpha_1$-antagonists have found clinical application in the symptomatic treatment of benign prostatic hyperplasia (BPH). $\alpha_1$-Adrenoceptor antagonists may be more efficacious than finasteride (see Chapter 27, Pharmacology of Reproduction) in the medical treatment of BPH. It has recently been discovered that three subtypes of the $\alpha_1$-receptor exist, termed $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1C}$. Evidence points to preferential expression of the $\alpha_{1A}$-receptor in genitourinary smooth muscle. **Tamsulosin** is a specific antagonist at $\alpha_{1A}$-receptors; this agent has little effect at $\alpha_{1B}$- or $\alpha_{1C}$-subtypes. The increased specificity of tamsulosin for $\alpha_{1A}$-receptors may decrease the incidence of orthostatic hypotension relative to that associated with prazosin and other nonsubtype selective $\alpha_1$-adrenoceptor antagonists.

Selective blockade of $\alpha_2$-autoreceptors by such drugs as **yohimbine** leads to increased release of norepinephrine, with subsequent stimulation of cardiac $\beta_1$-receptors and peripheral vasculature $\alpha_1$-receptors. $\alpha_2$-Selective antagonists also cause increased insulin release through blockade of $\alpha_2$-receptors in the pancreatic islets, which normally suppress insulin secretion. Yohimbine was used in the past to treat erectile dysfunction, but the recent development of such drugs as sildenafil and vardenafil have widely replaced its use.

## β-Adrenergic Antagonists

β-Adrenergic antagonists block the positive chronotropic and inotropic actions of endogenous catecholamines at $\beta_1$-receptors, resulting in decreased heart rate and myocardial contractility. These drugs do not decrease blood pressure in normal individuals, but they do decrease blood pressure in hypertensive patients. Long-term use of β-adrenoceptor blockers causes a fall in peripheral vascular resistance, although the mechanism of this effect remains unclear. The decreases in peripheral vascular resistance and cardiac output both contribute to the antihypertensive effect of these drugs. Nonselective β-adrenoceptor antagonists also block $\beta_2$-receptors in bronchial smooth muscle, which can cause life-threatening bronchoconstriction in patients with asthma or chronic obstructive pulmonary disease. In addition, nonselective β-blockade may mask symptoms of hypoglycemia in diabetic patients. For these reasons, selective inhibitors of $\beta_1$-adrenoceptors have been developed.

Pharmacologic antagonists at β-adrenergic receptors can be divided into nonselective β-antagonists, nonselective β- and $\alpha_1$-antagonists, partial agonists, and $\beta_1$-selective antagonists (Table 8-2). Selective blockers of $\beta_2$-adrenergic receptors do not have any clinical utility.

**Propranolol, nadolol,** and **timolol** interact with $\beta_1$- and $\beta_2$-receptors equally and do not block α-receptors. These agents are used in the treatment of hypertension and angina. Propranolol is extremely lipophilic; at therapeutic plasma levels, its CNS concentration is sufficiently high that sedation and decreased libido may result. An ocular formulation of timolol is used in the treatment of glaucoma.

**Labetalol** and **carvedilol** block $\alpha_1$-, $\beta_1$-, and $\beta_2$-receptors (Labetalol also acts as a weak partial agonist at $\beta_2$-receptors, but has a fivefold to 10-fold greater effect as a β-blocker). The $\alpha_1$-receptor blockade results in vasodilation, and the $\beta_1$-blockade prevents a reflex sympathetic increase in heart rate; both of these effects contribute to a decrease in blood pressure. Because labetalol may cause liver damage, liver function tests should be checked regularly in patients receiving this drug. Both labetalol and carvedilol are used to treat hypertension; the long-term advantages and disadvantages of these drugs relative to those of other β-blockers have not yet been elucidated.

**Pindolol** is a partial agonist at $\beta_1$- and $\beta_2$-receptors. The drug blocks the action of endogenous norepinephrine at $\beta_1$-receptors and is, therefore, useful in treating hypertension. As a partial agonist, pindolol also causes partial stimulation of $\beta_1$-receptors, leading to overall smaller decreases in resting heart rate and blood pressure than those caused by pure β-antagonists. Therefore, the drug may be preferable in hypertensive patients who have bradycardia or decreased cardiac reserve. **Acebutolol** is a partial agonist at $\beta_1$-adrenoceptors but has no effect at $\beta_2$-receptors. This agent is also used to treat hypertension.

**TABLE 8-2** Selectivity of β-Adrenoceptor Antagonists

| DRUG | NOTES |
|---|---|
| **Nonselective β-Adrenergic Antagonists** | |
| Propranolol | Short half-life |
| Nadolol | Long half-life |
| Timolol | Lipophilic—high CNS penetration |
| **Nonselective β- and $\alpha_1$-Antagonists** | |
| Labetalol | Also partial agonist at $\beta_2$-receptors |
| Carvedilol | Intermediate half-life |
| **β-Adrenergic Partial Agonists** | |
| Pindolol | β-nonselective |
| Acebutolol | $\beta_1$-selective |
| **$\beta_1$-Selective Adrenergic Antagonists** | |
| Esmolol | Short half-life (4 minutes) |
| Metoprolol | Intermediate half-life |
| Atenolol | Intermediate half-life |
| Celiprolol | Also agonist at $\beta_2$-receptors |

**Esmolol, metoprolol,** and **atenolol** are $\beta_1$-selective adrenergic antagonists. The elimination half-life is the main feature that distinguishes among these agents. Esmolol has an extremely short half-life (3–4 min); metoprolol and atenolol have intermediate half-lives (4–8 hours). Because of its short half-life, esmolol is used for emergency β-blockade, as in thyroid storm (see Chapter 25, Pharmacology of the Thyroid Gland). Clinical trials have suggested that β-blockers, especially metoprolol, prolong life expectancy in patients with moderate to mild congestive heart failure and in patients who have survived a first myocardial infarction (see Chapter 23, Integrative Cardiovascular Pharmacology). **Celiprolol** is a $\beta_1$-selective antagonist and $\beta_2$-selective agonist.

## Conclusion and Future Directions

Adrenergic pharmacology involves drugs that act at every step of adrenergic neurotransmission, from synthesis of catecholamines to stimulation of α- and β-receptors. The drugs discussed in this chapter are mainstays of therapy for depression, psychiatric disorders, hypertension, angina, shock, asthma, pheochromocytoma, and other conditions. The beneficial pharmacologic actions of these drugs, as well as their important side effects, can be deduced from knowledge of their molecular and cellular mechanisms of action and how these actions affect the processes of adrenergic neurotransmission. For example, gene cloning has recently identified three subtypes of $\alpha_1$-receptors and three subtypes of $\alpha_2$-receptors. The clinical relevance of these subtypes has not yet been determined, but the development of more specific agonists and antagonists may lead to more effective (and less toxic) therapies for hypertension and prostatic hypertrophy. Although a wide variety of β-blockers currently exist for clinical use, the functional consequence of using a particular β-blocker in a particular clinical situation has often not been delineated. Future research will determine, for example, whether use of a partial agonist is more efficacious in certain patient populations and the specific parameters for use of β-adrenoceptor antagonists in patients with congestive heart failure.

## Suggested Readings

Goldstein S. β-blockers in hypertensive and coronary heart disease. Arch Intern Med 1996;156:1268–1286. (*Reviews the clinical applications of β-blockers.*)

Goldstein S. Benefits of beta-blocker therapy for heart failure: Weighing the evidence. Arch Intern Med 2002;162:641–648. (*Reviews the basic science and clinical evidence for use of β-adrenoceptor antagonists in heart failure.*)

Kalkanis SN, Sloane D, Strichartz GR, Lilly LS. Cardiovascular drugs. In: Lilly LS, ed. Pathophysiology of Heart Disease. Philadelphia: Lippincott Williams & Wilkins, 1998. (*Good overview of cardiovascular actions of sympathomimetic amines and adrenergic receptor blockers.*)

Nelson HS. 1995. β-adrenergic bronchodilators. N Engl J Med 1995;333:499–506. (*Good review article on $\beta_2$-selective adrenergic agonists.*)

## Drug Summary Table Adrenergic Pharmacology

| | | | | |
|---|---|---|---|---|
| **INHIBITORS OF CATECHOLAMINE SYNTHESIS**<br>*Mechanism—Inhibit intermediate synthetic steps* | | | | |
| **α-Methyltyrosine** | Hypertension | Orthostatic hypotension<br>Sedation | | Inhibits tyrosine hydroxylase<br>Used only for pheochromocytoma-associated hypertension |
| **INHIBITORS OF CATECHOLAMINE STORAGE**<br>*Mechanism—Displace vesicular storage of catecholamines* | | | | |
| **Reserpine** | Hypertension | Sedation, depression | MAO inhibitors, history of depression | Inhibits monoamine vesicular uptake |
| **Guanethidine**<br>**Guanadrel** | Hypertension | Orthostatic hypotension | MAO inhibitors | Deplete neurotransmitter from vesicles |
| **Amphetamine** | Narcolepsy<br>Appetite suppressant | Restlessness, addiction<br>Rebound fatigue | MAO inhibitors, cardiovascular disease | Displaces neurotransmitter, inhibits MAO, stimulates α- and β-receptors |
| **Methylphenidate** | Attention-deficit hyperactivity disorder (ADHD) | Similar to amphetamine | MAO inhibitors, clonidine | Similar to amphetamine |
| **Ephedrine**<br>**Pseudoephedrine**<br>**Phenylpropanolamine** | Nasal congestion | Rebound congestion | Angle-closure glaucoma | Mixed-acting sympathomimetics |
| **INHIBITORS OF CATECHOLAMINE REUPTAKE**<br>*Mechanism—Block norepinephrine transporter (NET), potentiate catecholamine action* | | | | |
| **Cocaine** | Local anesthesia, abuse | Addiction | Pregnancy | Blocks DAT, NET, 5HTT (see Chapter 16) |
| **Imipramine**<br>**Amitriptyline** | Depression | Postural hypotension<br>Tachycardia | MAO inhibitors can cause serotonin syndrome | Block NET, serotonin transporter (5HTT) |
| **MONOAMINE OXIDASE (MAO) INHIBITORS**<br>*Mechanism—Inhibit MAO, increase catecholamine levels* | | | | |
| **Phenelzine**<br>**Iproniazid**<br>**Tranylcypromine** | Depression | All MAO inhibitors can cause sedation, postural hypotension | Coadministration of any MAO inhibitor with tyramine can cause hypertensive crisis. Coadministration with SSRIs and TCAs can cause serotonin syndrome | Nonselective, irreversible inhibitors of MAO-A and MAO-B |
| **Clorgyline** | Depression | | | Selective MAO-A inhibitor |
| **Brofaromine**<br>**Befloxatone**<br>**Moclobemide** | Depression | | | Selective, reversible MAO-A inhibitors |
| **Selegiline** | Parkinson's disease | | | Selective MAO-B inhibitor |
| **α-ADRENERGIC AGONISTS**<br>*Mechanism—Activate α-adrenergic receptors* | | | | |
| **Methoxamine** | Hypotension, shock | Bradycardia (vagal reflex) | | Systemic, $\alpha_1$-selective agonist |

| | | | | |
|---|---|---|---|---|
| **Phenylephrine**<br>**Oxymetazoline**<br>**Tetrahydrazoline** | Nasal congestion<br>Ophthalmic hyperemia | Rebound hypersensitivity | Hypertension | Topically administered α-agonists |
| **Clonidine**<br>**Guanabenz**<br>**Guanfacine** | Hypertension<br>Drug withdrawal | Bradycardia<br>↓ symptoms of hypoglycemia | Sedative CNS agents | $\alpha_2$-Selective agonists |
| **Methyldopa** | Hypertension | Hepatotoxicity (rare)<br>Hemolytic anemia | MAO inhibitors | Metabolized to methylnorepinephrine; acts as $\alpha_2$-agonist<br>Preferred antihypertensive agent in pregnancy |
| **β-ADRENERGIC AGONISTS**<br>*Mechanism—Activate β-adrenergic receptors* | | | | |
| **Isoproterenol** | Bronchoconstriction | Tachycardia | Angina | Nonselective β-agonist |
| **Dobutamine** | Congestive heart failure | ↑ Myocardial oxygen demand | Idiopathic hypertrophic subaortic stenosis (IHSS) | $\beta_1$-Selective agonist |
| **Metaproterenol**<br>**Terbutaline**<br>**Albuterol**<br>**Salmeterol** | Asthma | Skeletal muscle tremor<br>Tachycardia | | $\beta_2$-Selective agonists<br>Pulmonary delivery minimizes side effects<br>Salmeterol is long-acting |
| **α-ADRENERGIC ANTAGONISTS**<br>*Mechanism—Block α-adrenergic receptors* | | | | |
| **Phenoxybenzamine** | Pheochromocytoma | Postural hypotension | Shock | Irreversibly blocks α-receptors |
| **Phentolamine** | Pheochromocytoma | Postural hypotension | Renal impairment | Reversibly blocks α-receptors |
| **Prazosin**<br>**Terazosin**<br>**Doxazosin** | Hypertension<br>Benign prostatic hypertrophy | First-dose postural hypotension | TCAs may increase risk of postural hypotension | $\alpha_1$-Selective antagonists |
| **Tamsulosin** | Benign prostatic hypertrophy | Impaired ejaculation | | $\alpha_{1A}$-Selective antagonist |
| **Yohimbine** | Impotence | Tremors | | $\alpha_2$-Selective antagonist |
| **β-ADRENERGIC ANTAGONISTS**<br>*Mechanism—Block β-adrenergic receptors* | | | | |
| **Propranolol**<br>**Nadolol**<br>**Timolol** | All β-adrenergic antagonists can be used in:<br>Hypertension<br>Angina<br>Congestive heart failure<br>Esmolol is also used in thyroid storm | All β-adrenergic antagonists can cause:<br>Sedation<br>Decreased libido<br>Bradycardia<br>Asthma— cross-react with $\beta_2$ receptors<br>Diabetes—may ↓ symptoms of hypoglycemia<br>Labetalol can also cause liver damage | All β-adrenergic antagonists are contraindicated in:<br>Bradycardia<br>Heart block<br>Uncompensated heart failure<br>Pregnancy (2nd and 3rd trimesters) | Nonselective β-adrenergic antagonists<br>Timolol is used for glaucoma |
| **Labetalol**<br>**Carvedilol** | | | | Nonselective β-adrenergic antagonists; also antagonize $\alpha_1$ receptors |
| **Pindolol**<br>**Acebutolol** | | | | Nonselective partial β-agonists |
| **Esmolol**<br>**Metoprolol**<br>**Atenolol**<br>**Celiprolol** | | | | $\beta_1$-Selective antagonists<br>Celiprolol is also an agonist at $\beta_2$-receptors<br>Esmolol has a short (4 min) half-life |

# 9

# Local Anesthetic Pharmacology

Kalpana L. Mani and Gary R. Strichartz

**Introduction**
**Case**
**Physiology of Nociception**
- Transmission of Pain Sensation
  - *First Pain*
  - *Second Pain*
- Pain Perception
- Analgesia and Anesthesia

**Pharmacologic Classes and Agents**
- Chemistry of Local Anesthetics
  - *Aromatic Group*
  - *Amine Group*
- Mechanism of Local Anesthesia
  - *Anatomic Considerations*
  - *Voltage-Gated Sodium Channel*
  - *Other Receptors for Local Anesthetics*
- Pharmacokinetics of Local Anesthetics
  - *Systemic Absorption*
  - *Distribution*
  - *Excretion*
- Administration of Local Anesthetics
  - *Topical Anesthesia*
  - *Infiltration Anesthesia*
  - *Peripheral Nerve Blockade*
  - *Central Nerve Blockade*
  - *Intravenous Regional Anesthesia*
- Major Toxicities
- Individual Agents
  - *Ester-Linked Local Anesthetics*
  - *Amide-Linked Local Anesthetics*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

The word "anesthesia" comes directly from the Greek: "an" meaning without, and "aisthesis" meaning feeling or sensation. **Local anesthetics (LAs)** are a set of chemicals, with similar molecular structures, that can both inhibit the perception of sensations (importantly pain) and prevent movement. They are used in a variety of situations, ranging from topical application for burns and small cuts, to epidural blocks during obstetric procedures, to injections during dental care.

**Cocaine,** the first local anesthetic, comes from the leaves of the coca shrub (*Erythroxylon coca*). It was first isolated in 1860 by Albert Niemann, who noted its numbing powers. In 1886, Carl Koller introduced cocaine into clinical practice as a topical ophthalmic anesthetic. However, its addictive properties and toxicity prompted the search for substitutes. **Procaine,** the first of these substitutes, was synthesized in 1905. Known as *Novocain*, it is still used today, although less frequently than some more recently developed LAs.

*Local anesthetics block action potentials, which propagate impulses along neurons (see Chapter 5), by inhibiting voltage-gated sodium channels.* This effect is common to all excitable cell membranes. Voltage-gated sodium channels are responsible for depolarizing cell membranes in the generation and propagation of action potentials. By blocking this process, LAs prevent transmission of pain sensation to the central nervous system (CNS). LAs are not selective for pain fibers; they can also block other sensory, motor, and autonomic fibers, as well as action potentials in skeletal and cardiac muscle. This nonselective blockade can serve other useful functions (see Chapter 17, Pharmacology of Cardiac Rhythm), or can be a source of toxicity.

## Case

EM is a 24-year-old graduate student in organic chemistry. While working in the lab one evening, he spills a beaker of hydrofluoric acid (HF) in the fume hood. Although he reflexively jerks his hand away, some of the liquid falls on the fingertips of his left hand. EM initially feels a stinging pain, which increases in intensity and is followed by a burning, throbbing ache. Realizing the corrosiveness of the acid, EM immediately begins rinsing his hand with water and a magnesium sulfate solution (the magnesium chelates the toxic fluoride ions). About 10 minutes after the accident, he telephones 911 and is transported to the Emergency Department.

The resident notes that the HF has penetrated the nail beds of the affected fingers, and that EM is in severe pain. She commends him on his timely and appropriate actions, and decides on treatment with calcium gluconate (another fluoride chelator) to neutralize the remaining HF, in conjunction with a digital nerve block to reduce the pain. Lidocaine without epinephrine is injected into the fingers, followed by calcium gluconate. EM first notices a relief of the stinging, although the ache takes somewhat longer to fade. By the time his wounds are dressed and covered with plastic wrap, he cannot feel any sensation in his fingers. Over the next 2 weeks, EM's wounds heal spontaneously and the pain, now well controlled with ibuprofen, abates. He is able to plunge back into lab work, but his brush with serious injury affects him in an unforeseen way: he begins to contemplate applying to medical school.

## QUESTIONS

1. What is lidocaine's mechanism of action? To which broader class of drugs does it belong?
2. Why did EM's stinging pain arrive before the dull aching pain, and why did it resolve more quickly after lidocaine administration?
3. Why is epinephrine sometimes administered with lidocaine, and why was it not coadministered in this case?

## PHYSIOLOGY OF NOCICEPTION

**Nociception** is the activation of primary sensory nerve fibers (nociceptors) by noxious stimuli; that is, stimuli that are potentially tissue damaging. These include high temperatures, intense mechanical perturbations, and harsh chemicals. Nociceptors have free nerve endings located in the skin, deep soma, and viscera. Nociceptor cell bodies are located in the dorsal root ganglia close to the spinal cord or in the trigeminal ganglion for innervation of the face (Fig. 9-1). Nociceptors transmit impulses from the periphery to the dorsal horn of the spinal cord, where the information is subsequently processed through synaptic circuitry and transmitted to various parts of the brain. Thus, nociceptors are the first in a chain of neurons responsible for pain perception. Because nociceptors transmit information toward the brain, they are termed **afferent neurons.**

*Tissue damage is the primary stimulus for nociceptor activation.* Nociceptors do not transmit information about a breeze against one's skin or a firm touch, for example (the nerves that do this are termed tactile or low-threshold mechanoreceptors). Rather, nociceptors are activated when one touches a hot stove or slams a door on one's fingers (or spills acid on them). Nociceptors have receptors in their cell membranes for chemicals, such as **bradykinin,** that are released when nearby cells are injured. These transducing receptors convert noxious stimuli into "generator currents" that depolarize the neuron and can result in action potentials (Fig. 9-1).

For sensory stimuli whose intensity is above the nociceptor's threshold (e.g., above a certain temperature), the frequency of action potential generation increases as the stimulus intensity is elevated (Fig. 9-2). These action potentials travel along the axon and activate synapses on neurons in the dorsal horn of the spinal cord. This information is then further processed and projected to the brain. If impulses in the nociceptive afferents are sufficiently frequent, they are perceived as "painful." In a local (or segmental) circuit, nociceptive afferent axons also connect indirectly via interneurons to efferent (motor) neurons in the spinal cord, which then travel to the periphery and cause muscle movement. The motion of jerking one's hand away after touching a hot stove, which is a more complex response than the local circuit described above, is nevertheless initiated in this manner by nociceptors and mediated by spinal circuits.

## TRANSMISSION OF PAIN SENSATION

In their simplest form, neurons are composed of dendrites, a cell body, and an axon. Axons transmit information along the neuron from the cell body or from free nerve endings to dendrites, which synapse with other neurons. Depending on the diameter of the axons and their myelination status, axons are classified as **A-fibers, B-fibers,** or **C-fibers.** A-fibers and B-fibers are myelinated, while C-fibers are nonmyelinated (Table 9-1). Myelin is composed of the cell membranes of supportive cells in the nervous system, including Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system (CNS). In myelinated neurons, the supportive cell membrane (myelin) wraps many times around neuronal axons to form an electrically insulating sheath that greatly increases the velocity of impulse transmission.

The most important fibers for pain perception are the axons of afferent nociceptors, including the anatomically classified **Aδ-fibers** and **C-fibers.** The afferent nociceptors are comprised functionally of *thermal* nociceptors that are activated at temperatures above 45°C (C-fibers) or below 5°C (Aδ-fibers), *high-threshold mechanical* nociceptors that exclusively transmit information indicating injurious force on the skin (Aδ-fibers), and *polymodal* nociceptors that are activated by thermal, chemical, and mechanical stimuli (C-fibers) (Fig. 9-2).

### First Pain

The myelinated Aδ-fibers transmit impulses much faster than do the nonmyelinated C-fibers. An Aδ-fiber transmits impulses along its axon at a rate of 5–25 meters per second (m/s). In pain transmission, the Aδ-fibers are analogous to highways, while the C-fibers are akin to country lanes. In the former, the impulses race to their destination, while in the latter, impulses are restricted by a slower speed limit.

The Aδ-fibers transmit what is called **first pain.** First pain is transmitted quickly, is sharp ("pinprick-like") in quality, and is highly localized on the body. The density of Aδ-fibers is high on the fingertips, face, and lips but relatively low on the back (Fig. 9-3). Aδ-fibers require a weaker stimulus for excitation than do C-fibers.

### Second Pain

All C-fibers are nonmyelinated, and transmit action potentials at a rate of about 1 m/s. C-fiber nociceptors are predom-

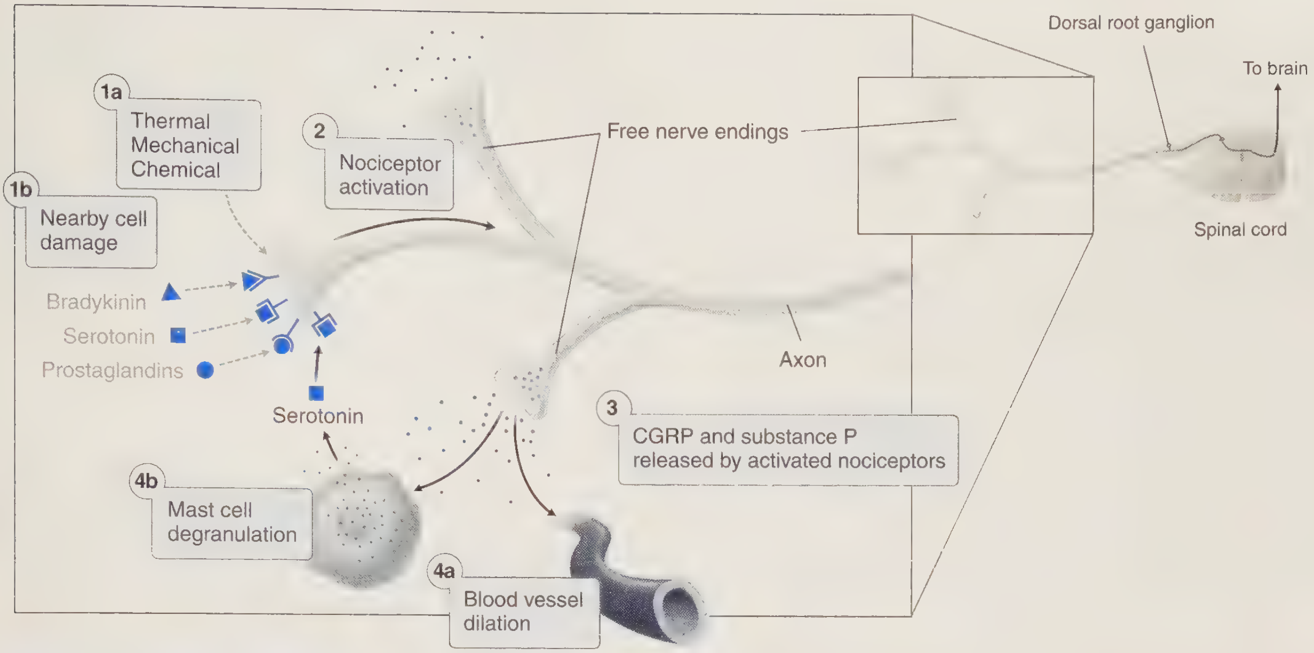


**Figure 9-1. Nociceptor Activation.** Nociceptors transmit pain information using a variety of mechanisms. Some receptors transduce noxious stimuli (thermal, mechanical, or chemical) into electric potentials. Other receptors are stimulated by chemicals that are released when nearby cells are injured (bradykinin, serotonin, prostaglandins). The release of $K^+$ from nearby damaged cells directly depolarizes nociceptor membranes. All of these stimuli cause nociceptor "sensitization," which decreases the threshold for activation. **1a.** A noxious stimulus leads to nociceptor activation and action potential generation **(2). 1b.** Concurrent nearby cell damage causes nociceptor sensitization. **3.** Activated nociceptors release molecules, including substance P and calcitonin gene related peptide (CGRP), that contribute to further sensitization and that initiate inflammatory responses to promote healing. For example: **4a.** Blood vessel dilation promotes white blood cell recruitment to the area; and **4b.** Mast cell degranulation releases histamine and serotonin, thus increasing sensitization.

inantly polymodal, which means that they can be activated by thermal, chemical, and mechanical noxious stimuli. Impulses in these C-fibers are responsible for what is called **second pain.** Second pain is slower in arriving but longer lasting; it feels dull, throbbing or burning, is only diffusely localizable, and endures after the stimulus ends. In the case presented above, EM experienced an initial stinging first pain transmitted by myelinated Aδ-fibers, and a later burning and throbbing second pain transmitted by unmyelinated C-fibers.

## PAIN PERCEPTION

Impulses generated in the skin by nociceptor activation are carried by Aδ- and C-fibers to the dorsal horn of the spinal cord. In the dorsal horn, the nociceptors form synapses with interneurons and second-order neurons. The second-order neurons travel in the lateral areas of the spinal cord and project mainly to the thalamus, a gray matter structure just superior to the brainstem. The thalamus has cells that project to the somatosensory cortex of the parietal lobe and to other areas of the cortex (Fig. 9-4). Pain perception is a complex process that normally results from the activation of nonnociceptive as well as nociceptive afferents, and can be altered depending on the situation, the person's state of mind, and other factors. The CNS uses efferent projections within the brain and spinal cord to modulate the incoming nociceptive signals, and thus to modify pain perception. For example, an athlete focused on an important game might not feel the pain of an injury as intensely until after the game is over. Her brain modulates the effect of the input so that the same stimulus is less painful at certain times than at others.

## ANALGESIA AND ANESTHESIA

The terms "analgesic" and "anesthetic" are frequently confused. **Analgesics** are specific inhibitors of *pain* pathways; whereas, **local anesthetics** are *nonspecific* inhibitors of peripheral sensory (including pain), motor, and autonomic pathways. Analgesics have actions at specific receptors on primary nociceptors and in the CNS (see Chapter 15, Pharmacology of Analgesia). For example, opioid analgesics activate opiate receptors, which signal the cells to increase potassium conductance in postsynaptic neurons and decrease calcium entry into presynaptic neurons. By these mechanisms, postsynaptic excitability and presynaptic transmitter release are reduced, and pain sensations are not transmitted as effectively to the brain (or within it). Importantly, the transmission of other sensations and motor information is not affected.

Local anesthetics act by a different mechanism. These agents inhibit conduction of action potentials in all afferent and efferent nerve fibers, usually in the peripheral nervous system (PNS). Thus, pain and other sensory modalities are not transmitted effectively to the brain, and motor impulses are not transmitted effectively to muscles in the periphery.

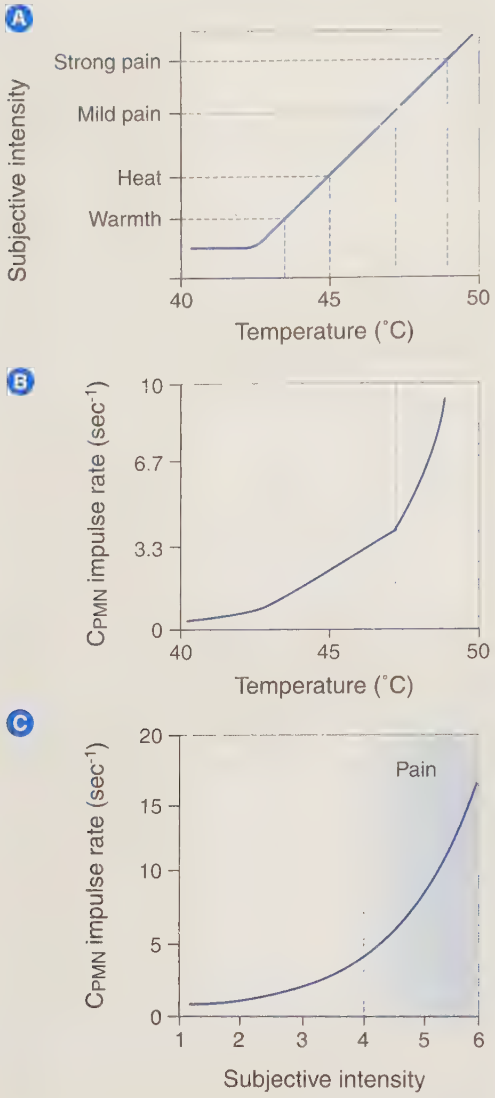


**Figure 9-2. Pain and Nociceptor Firing.** Most people find temperatures of 40–45°C to be warm to the touch, temperatures of 46–47°C to be slightly uncomfortable, and temperatures greater than 47°C to be painful. **A.** Human volunteers subjectively rate sensations of warmth, heat, mild pain, and strong pain as a function of temperature. **B.** Correlation between the firing rate of C polymodal nociceptors (PMN) and temperature. The rate of C fiber discharge increases significantly above 47°C (pain threshold). **C.** The C fiber response accelerates as pain increases: the increasing intensity of pain correlates with an increase in the frequency of firing. Thus, the higher the temperature, the more frequently the nociceptors fire and the more pain is felt.

# PHARMACOLOGIC CLASSES AND AGENTS

## CHEMISTRY OF LOCAL ANESTHETICS

Local anesthetics act regionally to block nerve fiber conduction. They act primarily by *inhibiting voltage-gated sodium channels in nerve cell membranes.* To understand the pharmacology of local anesthetics, it is helpful to understand their structures. Generally, all local anesthetics have three structural domains: an aromatic group, an amine group, and an ester or amide linkage connecting these two groups (Fig. 9-5). As discussed below, the structure of the aromatic group influences the hydrophobicity of the drug, the nature of the amine group influences the rate of onset and potency of the drug, and the structure of the amide or ester group influences the duration of action and side effects of the drug. Clinically, local anesthetics are classified as **ester-linked LAs** or **amide-linked LAs.** Because all LAs share similar properties, the next section highlights the general principles of LA pharmacology. Specific LA agents are briefly discussed at the end of the chapter.

### Aromatic Group

All local anesthetic molecules contain on one end an aromatic group that gives the molecule its hydrophobic character. Adding substituents on the aromatic ring (or sometimes elsewhere) can alter the hydrophobicity of the drug.

Biological membranes have a hydrophobic interior because of their lipid bilayer structure. The hydrophobicity of a LA drug affects the ease with which the drug passes through nerve cell membranes to reach its target site, which is the cytoplasmic side of the voltage-gated sodium channel (Fig. 9-6). In general, as the hydrophobicity of a series of drugs increases, the permeability of the drugs through the cell membrane also increases. At a certain hydrophobicity this relationship reverses, however, and a further increase in hydrophobicity results in a decrease in permeability. There are two reasons for this somewhat paradoxical behavior. First, molecules with very little hydrophobicity do not partition into the membrane at all, because their solubility in the lipid bilayer is so low. Rather, such molecules are restricted to the polar aqueous extracellular environment and never reach a location in the membrane from which they can diffuse to the cytoplasmic side of the membrane. Molecules that are very hydrophobic, on the other hand, partition strongly into the cell membrane and remain there. Although such molecules are concentrated in the cell membrane, they dissociate from the membrane very slowly because of the hydrophobic interactions that stabilize them there. In other words, their free energy of partitioning into the membrane is so great that they are essentially trapped. Neither of these two extremes is conducive to effective anesthetic action. *An effective local anesthetic must partition into, diffuse across, and finally dissociate from the membrane; the compounds most likely to do so have a moderate hydrophobicity.*

The LA binding site on the sodium channel also contains hydrophobic residues. Therefore, more hydrophobic drugs bind more tightly to the target site, increasing the potency of the drug. However, because of the practical need for the drug to diffuse across several membranes in order to be delivered to the target site, LAs with moderate hydrophobicity are the clinically most effective forms. Excessively hydrophobic drugs remain in the first membrane that is encountered, never reaching the target site despite their high affinity for that site.

### Amine Group

The tertiary amine of a local anesthetic molecule can exist in either the protonated (positively charged) form, also known

**TABLE 9-1** Types of Peripheral Nerve Fibers

| FIBER TYPE | MYELINATED | DIAMETER (μm) | CONDUCTION VELOCITY (m/s) | FUNCTION | SENSITIVITY TO LIDOCAINE |
|---|---|---|---|---|---|
| Aα, Aβ | Yes | 6–22 | 10–85 | Motor and proprioception (pressure, touch, position) | +, ++ |
| Aγ | Yes | 3–6 | 15–35 | Muscle tone | ++ |
| Aδ | Yes | 1–4 | 5–25 | First pain and temperature | +++ |
| B | Yes | < 3 | 3–15 | Vasomotor, visceromotor, sudomotor, pilomotor | ++++ |
| C (sympathetic) | No | 0.3–1.3 | 0.7–1.3 | Vasomotor, visceromotor, sudomotor, pilomotor | ++++ |
| C (dorsal root) | No | 0.4–1.2 | 0.1–2.0 | Second pain and temperature | ++++ |

Each peripheral nerve fiber type is responsible for transmitting one or more specific modalities. For example, the nociceptors (Aδ and C dorsal root fibers) are responsible for transmitting pain and temperature sensations. These fibers are not activated by pressure, light touch, or position changes. Myelin is an insulator that allows impulses to be conducted at faster speeds along axons. The nonmyelinated C-fibers have a slower conduction velocity than do the myelinated fibers. The different fiber types are affected by local anesthetics with different sensitivities.

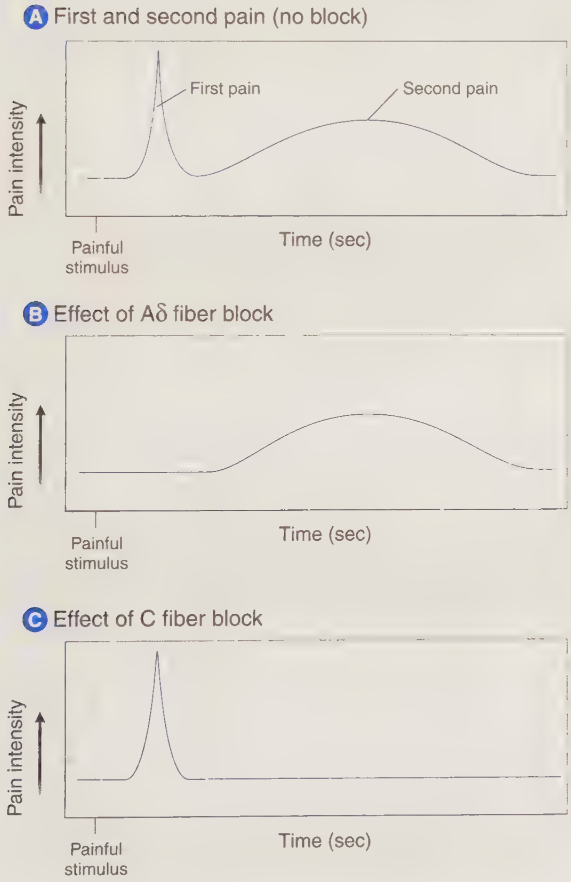


**Figure 9-3. First and Second Pain.** First pain, which is transmitted by Aδ-fibers, is sharp and highly localizable. Second pain, which is transmitted by C-fibers, is slower in arriving, duller, and longer lasting **(A)**. First pain can be blocked by selective blockade of Aδ-fibers **(B)**, and second pain can be blocked by selective blockade of C-fibers **(C)**. Because Aδ-fibers are more susceptible than C-fibers to blockade by local anesthetics, first pain often disappears at concentrations of anesthetic lower than that required to eliminate second pain.

as the conjugate acid, or the deprotonated (neutral) form, also known as the conjugate base.

The pKa is the pH at which the concentrations of a base and its conjugate acid are equal. LAs are weak bases; their pKa values range from about 8 to 10. Thus, at the physiologic pH of 7.4, both the protonated form and the neutral form exist in solution. As the pKa of a drug increases, a greater fraction of molecules exists in solution in the protonated form at physiologic pH (see Chapter 1, Drug–Receptor Interactions). Protonation and deprotonation reactions are very rapid in solution, but drugs in membranes are protonated and deprotonated more slowly.

The neutral forms of LAs cross membranes much more easily than do the positively charged forms. However, the positively charged forms bind with much higher affinity to the drugs' target binding site, which is located on the voltage-gated sodium channel near the intracellular opening of the channel. This is why moderately hydrophobic weak bases are so effective as local anesthetics. At physiologic pH, a significant fraction of the weak base molecules are in the neutral form that, because of its moderate hydrophobicity, can cross membranes to enter nerve cells. Once the drug is inside the cell, it can then rapidly gain a proton, become positively charged, and bind with higher affinity to the sodium channel.

The major path by which protons reach the drug molecules bound to the $Na^+$ channel is through the channel's pore to the extracellular environment. The ion pore of the channel runs through the membrane between the outside and the inside of the cell. If the extracellular pH is more acidic than the intracellular pH, there is a higher chance that the drug will become protonated at its site of binding to the channel. Once protonated, the drug dissociates much more slowly from the channel. The pH inside the cell does not have an important effect on the protonation state of drug molecules that are already bound to the channel; this lack of effect is thought to be attributable to the orientation of the drug within the channel. Some nonionizable drugs, such as benzocaine, are permanently neutral but are still able to block sodium channels and action potentials. For these drugs, however, the block is weak and does not depend on extracellular pH.

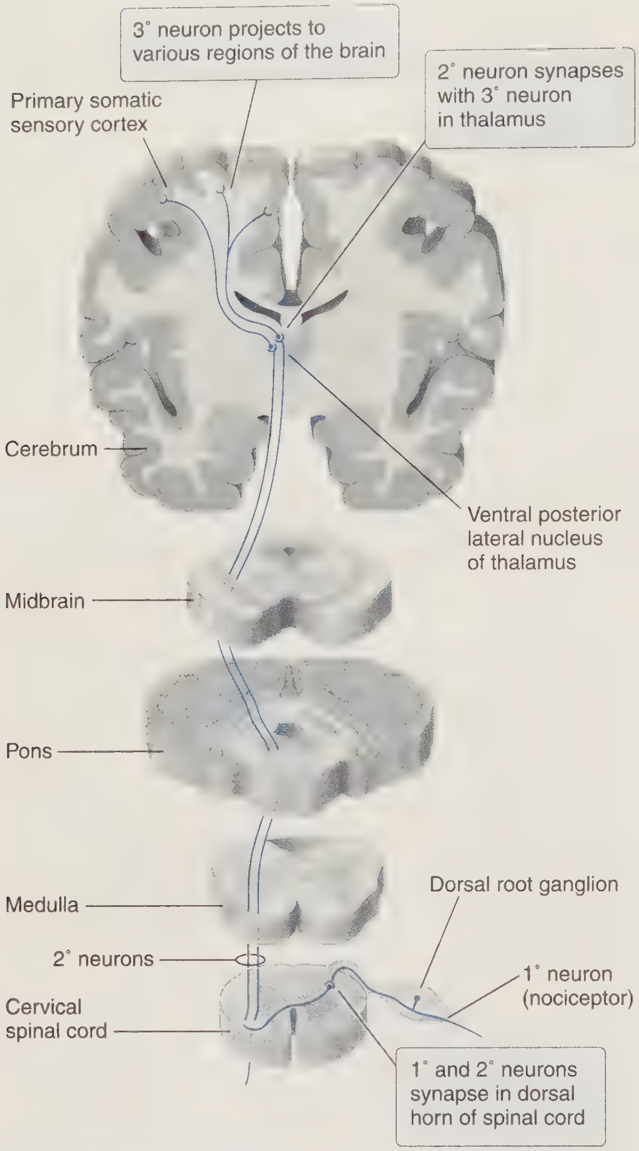

3° neuron projects to various regions of the brain
2° neuron synapses with 3° neuron in thalamus
Primary somatic sensory cortex
Cerebrum
Ventral posterior lateral nucleus of thalamus
Midbrain
Pons
Medulla
Dorsal root ganglion
2° neurons
1° neuron (nociceptor)
Cervical spinal cord
1° and 2° neurons synapse in dorsal horn of spinal cord

**Figure 9-4. Pain Pathways.** Primary (1°) nociceptors have cell bodies in the dorsal root ganglion, and synapse with secondary (2°) afferent neurons in the dorsal horn of the spinal cord. Primary afferents use the neurotransmitter glutamate. The 2° afferents travel in the lateral areas of the spinal cord and eventually reach the thalamus, where they synapse with tertiary (3°) afferent neurons. The processing of pain is complex, and 3° afferents have many destinations including the somatic sensory cortex (localization of pain) and the limbic system (emotional aspects of pain).

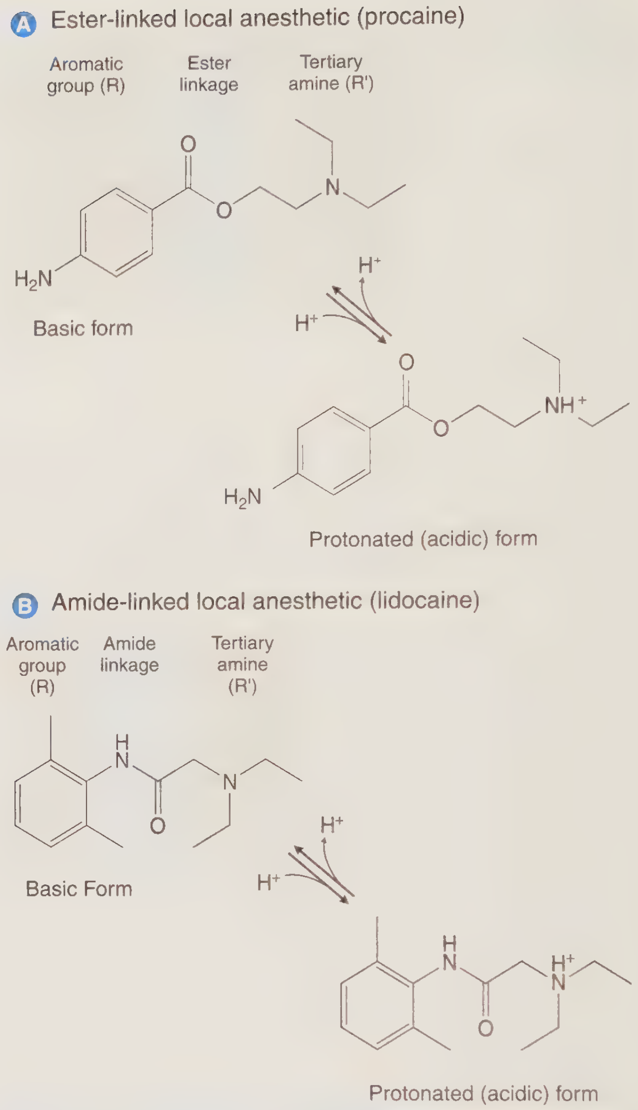

A Ester-linked local anesthetic (procaine)
Aromatic group (R)
Ester linkage
Tertiary amine (R')
O
O
N
H2N
Basic form
H+
H+
O
O
NH+
H2N
Protonated (acidic) form
B Amide-linked local anesthetic (lidocaine)
Aromatic group (R)
Amide linkage
Tertiary amine (R')
H
N
N
O
Basic Form
H+
H+
H
N
H+
N
O
Protonated (acidic) form

**Figure 9-5. Prototypical Local Anesthetics.** Procaine **(A)** and lidocaine **(B)** are considered the prototypical ester-linked and amide-linked local anesthetics, respectively. Local anesthetics have an aromatic group on one end and a tertiary amine on the other end of the molecule; these two groups are connected by an ester (-RCOOR′) or amide (-RHNCOR′) linkage. In solution at high pH, the equilibrium between the basic (neutral) and acidic (charged) forms of a local anesthetic favors the basic form. At low pH, the equilibrium favors the acidic form. At intermediate (physiological) pH, nearly equal concentrations of the basic and acidic forms are present. Generally, ester-linked local anesthetics are easily hydrolyzed to a carboxylic acid (RCOOH) and an alcohol (HOR′) in the presence of water and esterases. In comparison, amides are far more stable in solution. Consequently, amide-linked local anesthetics generally have a longer duration of action than do ester-linked anesthetics.

## MECHANISM OF LOCAL ANESTHESIA

### Anatomic Considerations

The peripheral nerve is composed of a collection of different types of nerve fibers (A-, B-, and C-fibers) surrounded by three protective membranes, or "sheaths": the epineurium, perineurium, and endoneurium. Local anesthetics must pass through these sheaths (presenting the same permeation-limiting barriers as the nerve cell membranes considered above) before they can reach the neuronal membranes (Fig. 9-7). The sheaths are made up of connective tissue and cellular membranes. LAs are injected outside the most external sheath, the epineurium, to avoid mechanical needle damage to the nerve, but the major barrier to LA penetration into the nerve is the perineurium, an epithelial-like tissue. Recall that LAs affect not only nociceptors but also other afferent and efferent, somatic and autonomic nerve fibers. All of these fibers are con-

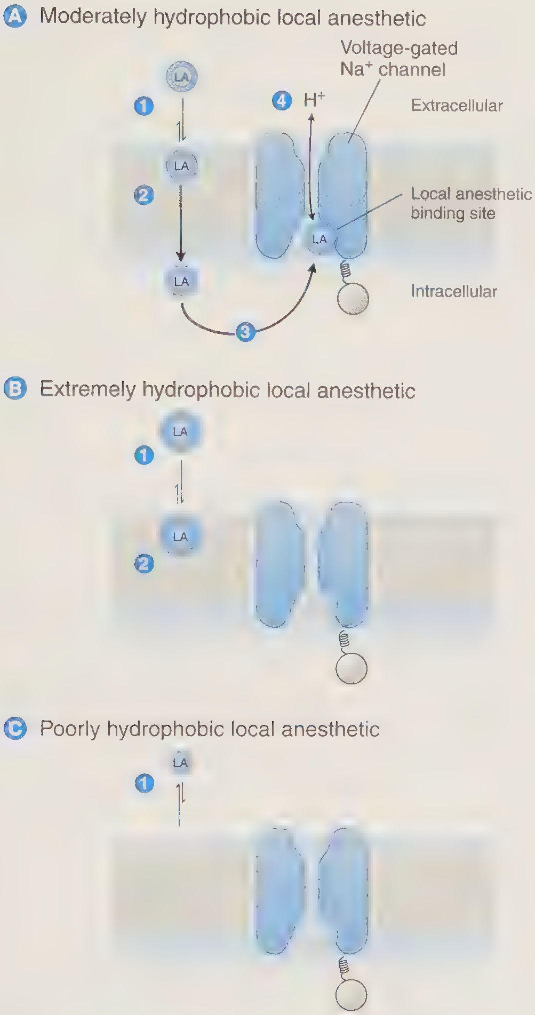

A Moderately hydrophobic local anesthetic
Voltage-gated Na⁺ channel
1
LA
4 H⁺
Extracellular
LA
2
Local anesthetic binding site
LA
LA
Intracellular
3
B Extremely hydrophobic local anesthetic
LA
1
LA
2
C Poorly hydrophobic local anesthetic
LA
1

**Figure 9-6. Local Anesthetic Hydrophobicity, Diffusion and Binding.** Local anesthetics act by blocking the cytoplasmic side of the voltage-gated $Na^+$ channel. The hydrophobicity of a local anesthetic determines how efficiently it diffuses across lipid membranes and how tightly it binds to the $Na^+$ channel, and, therefore, governs its potency. **A.** Moderately hydrophobic local anesthetics (LAs) are the most effective agents: (1) Neutral LA adsorbs to the extracellular side of the neuronal cell membrane; (2) LA diffuses through the cell membrane to the cytoplasmic side; (3) LA diffuses and binds to its binding site on the voltage-gated sodium channel; (4) once bound, LA can switch between its neutral and protonated forms by binding and releasing extracellular protons. **B.** Extremely hydrophobic LAs become trapped in the lipid bilayer: (1) Neutral LA adsorbs to the neuronal cell membrane (2) where it is so stabilized that it cannot dissociate from or translocate across the membrane. **C.** Poorly hydrophobic LAs are unable to cross the lipid bilayer efficiently: (1) Neutral LA cannot adsorb to or enter the neuronal cell membrane because it is very stable in the extracellular solution and has a very high activation energy for entering the hydrophobic membrane.

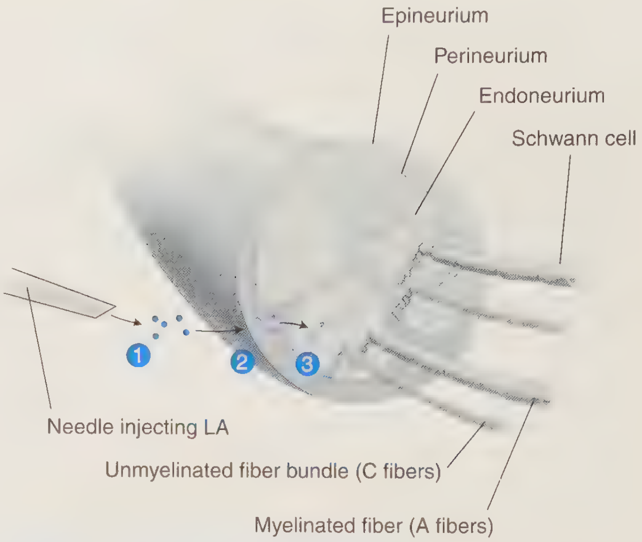


**Figure 9-7. Peripheral Nerve Anatomy.** 1. Local anesthetics (LAs) are injected or otherwise applied outside the peripheral nerve epineurium (the outermost sheath of connective tissue containing blood vessels, adipose tissue, fibroblasts, and mast cells). 2. LA molecules must cross the epineurium to reach the perineurium, another epithelial membrane, which organizes nerve fibers into fascicles. The perineurium is the most difficult layer for local anesthetics to penetrate, because of the tight junctions between its cells. 3. LAs then pass through the endoneurium, which invests the myelinated and unmyelinated fibers, Schwann cells, and capillaries. Only LAs that have passed through these three sheaths can reach the neuronal membranes, where the voltage-gated sodium channels reside. Clinically, a high concentration of local anesthetic must be applied because only a fraction of the molecules reach the target site.

tained within a peripheral nerve, and conduction in all fibers can be blocked by local anesthetics. If the peripheral nerve is considered to represent a multilane road, then each fiber type can be considered a lane in this road. A blockade across the whole road (the blockade of a local anesthetic) will stop traffic in all lanes in both directions. This is why, in the introductory case, EM experienced not only loss of pain sensation, but also a more complete block of all sensation in his digits.

In general, more proximal regions of the body (shoulder, thigh) are innervated by axons traveling relatively superficially in a peripheral nerve, while more distal regions (hands, feet) are innervated by axons traveling closer to the core of the nerve. Because local anesthetics are applied to the outside of a peripheral nerve, external to the epineurium, the axons innervating more proximal areas are usually reached first by the local anesthetic. Consequently, *the anatomic progression of functional block shows that proximal areas are numbed before distal areas.* For example, if a nerve block is applied in the brachial plexus, the shoulder and upper arm are numbed before the forearm, hand, and fingers (Fig. 9-7).

During the onset of local anesthesia, different fiber types within a peripheral nerve are also blocked at different times. The general order in which functional deficits occur is as follows: first pain, second pain, temperature, touch, proprioception (pressure, position, or stretch), and finally skeletal muscle tone and voluntary tension. This phenomenon is referred to as **differential functional blockade.** Because motor function is often the last ability to be lost, it is possible for some LAs to block nociception with relatively little effect on motor transmission. The concentration of local anesthetic required to block sensory impulses without inducing a large motor blockade varies for different agents. Using lidocaine, for example, it is difficult to block Aα-fibers without also blocking Aγ-motor fibers (Table 9-1); in contrast, epidural bupivacaine can achieve sensory block at low concentrations without significant motor block.

Conduction failure seems to be a function of axon type, drug concentration, length of exposed nerve fiber, and frequency of incoming impulses. In the introductory case, recall that EM's first pain was blocked before his second pain, and that block of both preceded the loss of his other sensory modalities. Clinically, if a patient is still able to feel the sharp pain of a pinprick, then the degree of anesthesia is unlikely to be sufficient to block the transmission of long-lasting second pain.

## Voltage-Gated Sodium Channel

Local anesthetics prevent impulse transmission by blocking individual sodium channels in neuronal membranes. The sodium channel exists generally in three conformational states: open, inactivated, and resting. In going from the resting to the open state, the channel also moves through several transient "closed" conformations. The resting neuronal membrane potential is −60 to −70 millivolts (mV). At this potential, the channels are in equilibrium between the resting state (majority) and the inactivated state (minority). During an action potential, the resting channels move through several closed conformations and finally open briefly to allow sodium ions to enter the cell. This sodium influx results in depolarization of the membrane. After approximately 1 millisecond, the open channel closes spontaneously to the inactivated conformation. This halts the influx of sodium, allowing membrane repolarization. The inactivated state of the channel returns slowly to the resting state in the repolarized membrane, largely defining the time-course of the refractory period. During the absolute refractory period, there are so few $Na^+$ channels in the resting state that, even if all of the resting-state channels were simultaneously activated to the open state, threshold would not be reached. Thus, no new action potentials can be generated during this period of time (Fig. 9-8A).

### 1. Modulated Receptor Hypothesis

The different conformational states of the sodium channel (resting, various closed, open, and inactivated) bind to local anesthetics with different affinities. In other words, the target binding site for local anesthetics (i.e., the sodium channel) is modulated (has different structures and, therefore, different affinities for LA molecules) by the neuronal membrane potential. This concept is known as the **modulated receptor hypothesis** (Fig. 9-8B and Table 9-2).

*Local anesthetics have a higher affinity for the open and inactivated states of the sodium channel than for the resting state.* However, local anesthetics also bind with high affinity to the intermediate closed conformational states through which the channel must progress to change from the resting to the open state. This binding of drug to the closed states seems to restrict the conformational changes of the sodium channel, so that a drug-bound channel cannot undergo the full range of conformational changes necessary to open the channel. Although the LA binds at a site near the cytoplasmic opening of the pore, the predominant molecular mechanism of channel inhibition is not the physical occlusion of the pore, but rather the restriction of activation of the channel.

For a drug-bound channel to reopen, the LA must dissociate from the channel and, thus, allow the channel to return to its resting state. This dissociation of drug (the rate of which varies among the different LAs) is slower than the normal recovery of the inactivated to the resting channel conformation in the absence of LA. Thus, LAs extend the refractory period of the neuron by delaying the inactivated channel's return to the resting state by about 50-fold to 100-fold. At high concentrations, LAs bind to a sufficient number of low-affinity resting channels that impulse conduction is prevented altogether.

### 2. Tonic and Phasic Inhibition

The differential affinity of local anesthetics for different states of the voltage-gated sodium channel has an important pharmacologic consequence: the degree of inhibition of sodium current by the LA depends on the frequency of impulses in the nerve. When there are long intervals between action potentials, the level of inhibition of each impulse is the same, and the inhibition is said to be tonic. When the intervals between action potentials are short, the level of inhibition increases with each successive impulse, and the inhibition is said to be phasic, or use dependent (Fig. 9-9).

**Tonic inhibition** occurs when the time between action potentials is long compared to the time for dissociation of the LA from the sodium channel. Assume, for example, that before an action potential arrives, an equilibrium has been established where 5% of the sodium channels are bound by local anesthetic molecules. When an action potential arrives, the other 95% of the channels are available to open and subsequently to inactivate. During the impulse, some of these channels become bound by local anesthetic molecules. However, there is sufficient time before the next impulse arrives for many of the LA-sodium channel complexes to dissociate and for those channels to return to the resting state. Thus, before the next action potential arrives, the 5% binding equilibrium is re-established. The next action potential will, therefore, be blocked to exactly the same extent as the previous one.

**Phasic inhibition** occurs when there is not enough time between action potentials for this equilibrium to be re-established. Rapidly arriving action potentials cause resting sodium channels to open and then inactivate. During an impulse, some of these channels are bound by LAs. However, because there is not enough time between impulses for all of the newly formed LA-sodium channel complexes to dissociate, only some of the channels are able to return to the resting state. With each arriving action potential, more and more channels are blocked until a new steady state of LA-sodium channel binding is reached. This is the phenomenon of phasic, or use-dependent, inhibition. As more of the channels are converted to LA-bound complexes, fewer and fewer channels are available to open when the next action potential arrives. Consequently, *action potential conduction is increasingly inhibited at higher frequencies of impulses.*

The clinical importance of this phenomenon is that tissue injury or trauma causes nociceptors in the area of injury to fire at high rates. Therefore, application of a local anesthetic will tend to block local nociceptors in a phasic manner, inhibiting pain transmission to a greater extent than other local sensory or motor impulses that are blocked only tonically.

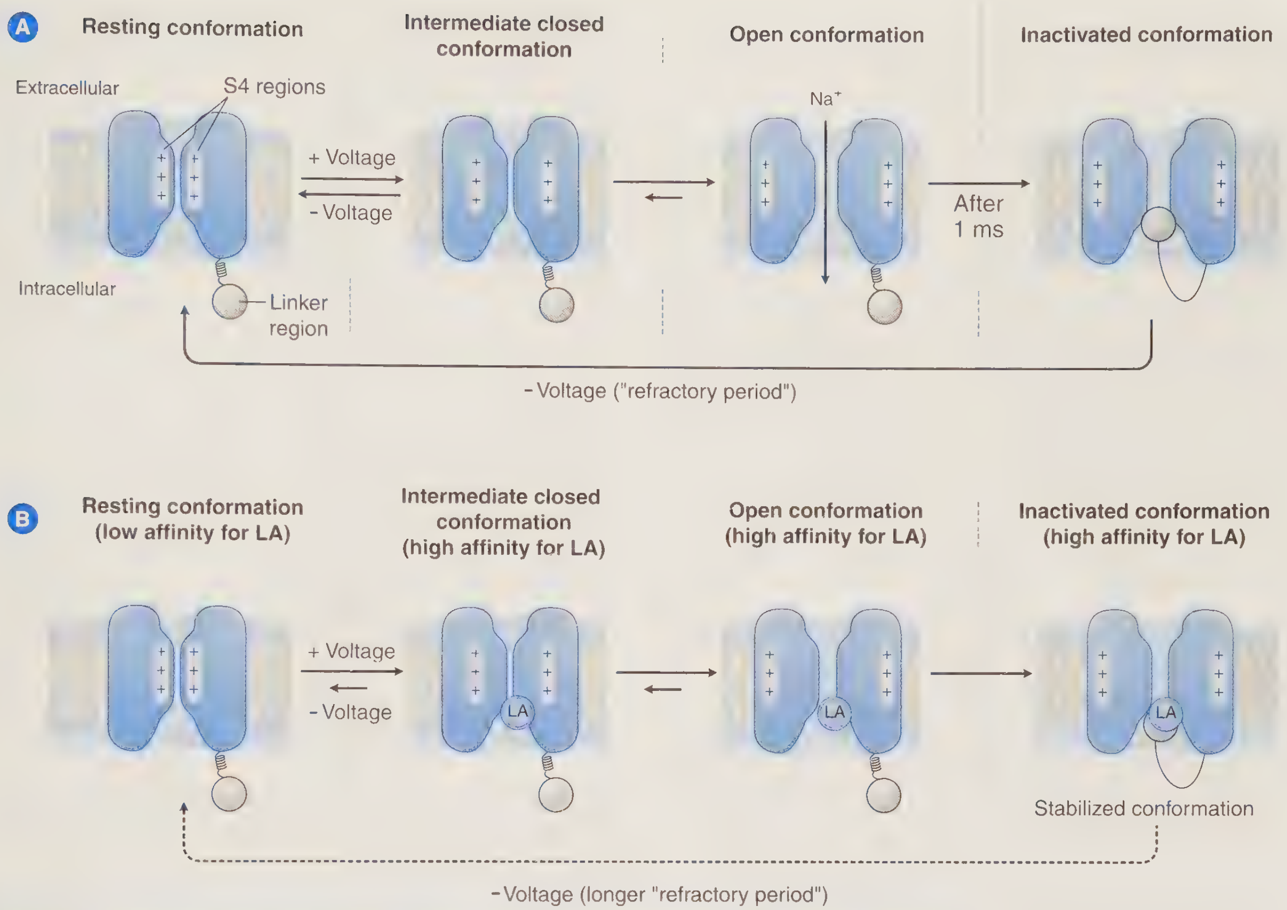
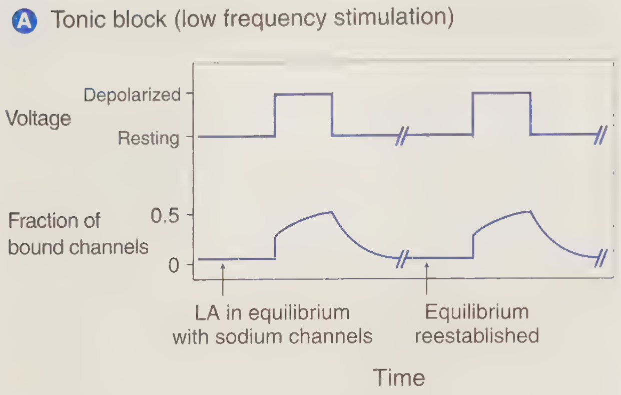

A
Resting conformation
Intermediate closed conformation
Open conformation
Inactivated conformation
Extracellular
S4 regions
Na+
+ Voltage
- Voltage
After 1 ms
Intracellular
Linker region
- Voltage ("refractory period")
B
Resting conformation (low affinity for LA)
Intermediate closed conformation (high affinity for LA)
Open conformation (high affinity for LA)
Inactivated conformation (high affinity for LA)
+ Voltage
- Voltage
LA
LA
LA
Stabilized conformation
- Voltage (longer "refractory period")

Figure 9-8. Local Anesthetic Binding to Different Conformations (states) of the Sodium Channel. **A.** The sodium channel is composed of one polypeptide chain that has four repeating units. One region, known as the S4 region, has many positively charged amino acids (lysine and arginine). These residues give the channel its voltage dependence. At rest, the pore is closed. When the membrane is depolarized, the charged residues move in response to the change in the electric field. This results in several conformational changes (intermediate closed states) that culminate in channel opening. After about 1 ms (the channel open time), the 3–4 amino acid "linker region" plugs the open channel, yielding the inactivated conformation. The inactivated conformation returns to the resting state only when the membrane is repolarized; this conformational change involves the return of the S4 region to its original position and the expulsion of the linker region. The time required for the channel to return from the inactivated state to the resting state is known as the refractory period; during this period, the sodium channel is incapable of being activated. **B.** The binding of local anesthetic (LA) alters the properties of the intermediate forms assumed by the sodium channel. Sodium channels in any of the conformations (resting, closed, open, or inactivated) can bind local anesthetic molecules, although the resting state has a low affinity for LA, while the other three states have a high affinity for LA. LA can dissociate from the channel–LA complex in any conformational state, or the channel can undergo conformational changes while associated with the LA molecule. Ultimately, the channel–LA complex must dissociate, and the sodium channel must return to the resting state to become activated. LA binding extends the refractory period, including both the time required for dissociation of the LA molecule from the sodium channel, and the time required for the channel to return to the resting state.

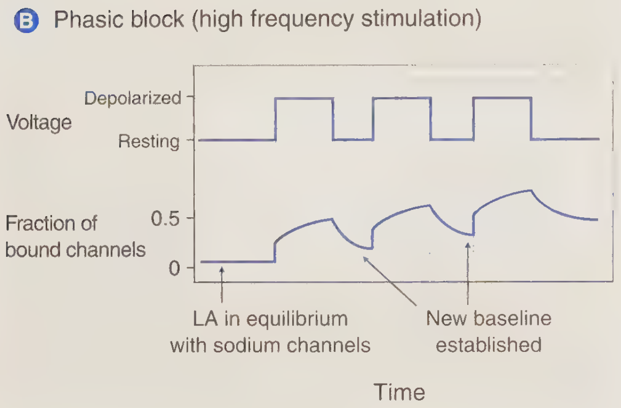
**TABLE 9-2** Modulated Receptor Hypothesis

| CHANNEL STATE | AFFINITY FOR LA | RELATIVE EFFECT ON CHANNEL |
|---|---|---|
| Resting | Low | Prevents channel opening (only at high LA concentrations) |
| Closed (several) | High | Prevents channel opening (major effect) |
| Open | High | Blocks channel pore (minor effect) |
| Inactive | High | Extends refractory period (major effect) |

The voltage-gated sodium channel can assume several different conformations. Local anesthetics have differing affinities for different conformations of the channel; this differential affinity alters the kinetics of sodium channel activation (see Fig. 9-9).



A Tonic block (low frequency stimulation)



B Phasic block (high frequency stimulation)

**Figure 9-9. Tonic and Phasic (Use-Dependent) Inhibition.** **A.** In tonic block, depolarizations occur with low frequency, and there is sufficient time between depolarizations for equilibrium binding of local anesthetic (LA) molecules to the various states of the sodium channel to be re-established. When a depolarization occurs, resting channels (which have low affinity for LA) are converted into open channels and inactivated channels (which have high affinity for LA). Thus, there is an increase in the number of LA-bound channels. Once the depolarization ends, there is enough time before the next depolarization for equilibrium between LA molecules and sodium channels to be re-established, and virtually all of the channels return to the resting and unbound state. **B.** In phasic block, depolarizations occur with high frequency, and there is not sufficient time between depolarizations for equilibrium to be re-established. After each depolarization, a new baseline is established that has more LA-bound channels than the previous baseline, leading eventually to conduction failure. Because high-frequency stimulation of nociceptors occurs in areas of tissue damage, phasic (use-dependent) block causes actively firing nociceptors to be more effectively inhibited than nerve fibers that are only occasionally firing. The frequency dependence of phasic block depends on the rate at which LA dissociates from its binding site on the channel.

## Other Receptors for Local Anesthetics

In addition to blocking sodium channels, local anesthetics can have a wide range of other biochemical and physiologic effects. LAs can interact with potassium channels, calcium channels, ligand-gated channels, such as the nicotinic acetylcholine receptor, and several G protein-coupled receptors (including muscarinic cholinergic receptors, β-adrenergic receptors, and receptors for substance P). LAs can also uncouple some G proteins from their cell surface receptors, and thus, inhibit signal transduction. In most cases, these effects are not significant because LAs have lower affinity for these other receptors than for the sodium channel. But for some types of local anesthetics in some clinical situations, these alternate targets may have important therapeutic and toxic consequences.

For example, in spinal anesthesia, a high concentration of local anesthetic is injected into the cerebrospinal fluid, from which the LA then diffuses into the spinal cord. **Substance P** is a peptide neurotransmitter in the dorsal horn of the spinal cord that modulates the transmission of nociceptive impulses between the primary and secondary afferent neurons (see above). Substance P is released by the presynaptic terminal of the primary afferent nociceptor, diffuses across the synaptic cleft, and binds to its NK1 receptor on the secondary afferent neuron. The binding of substance P to NK1 sensitizes the secondary neuron, increasing the likelihood that action potentials will be sent to the brain. LA binding to the NK1 receptor on the secondary neuron inhibits substance P binding. The result, an increased pain threshold, enhances the analgesia that results from the sodium channel-blocking effects of the LA.

## PHARMACOKINETICS OF LOCAL ANESTHETICS

After administration by injection or topical application, local anesthetics travel to their sites of action mainly through diffusion. Along the way, LA molecules are also taken up by the local tissues and removed by the local circulation. The amount of drug diverted into the local circulation depends on the vascularity of the tissue. In the blood, some types of local anesthetics bind rapidly and reversibly to plasma proteins and to erythrocytes. Local anesthetics are transported to and distributed in various tissues in the body. Ester-linked LAs are metabolized by local and systemic esterases, while amide-linked LAs are metabolized by cytochrome P450 enzymes in the liver. Metabolites of both types are cleared from the body by renal excretion.

### Systemic Absorption

The amount of local anesthetic that enters the systemic circulation and the potency of the LA together determine the systemic toxicity of the agent. Ideally, systemic absorption is minimized to avoid unnecessary toxicities. The vascularity of the injection site, the drug concentration, the addition of a vasoconstrictor, and properties such as the viscosity of the injected solution all influence the rate and extent of systemic absorption of local anesthetics. Absorption is greater from densely perfused tissues or following multiple administrations. For example, intratracheal administration of vaporized local anesthetic leads to quick and nearly complete systemic absorption because of local anesthetic contact with the highly perfused lung parenchyma.

Vasoconstrictors (such as epinephrine) are often administered together with many short-acting or medium-acting local anesthetics. These adjunctive agents reduce blood flow to the area of injection by causing the smooth muscles of the vessels to contract, and, therefore, slow the rate of removal of the LA. In doing so, vasoconstrictors both increase the concentration of anesthetic around the nerve and decrease the maximal concentration that is reached in the systemic circulation. The former effect enhances the duration of action of the LA, and the latter effect decreases the LA's systemic toxicity. However, vasoconstriction can also lead to tissue hypoxia and damage if the oxygen supply to the area is reduced too severely. Thus, *vasoconstrictors are not used with LAs for blockade of sensation in the extremities, because there is limited circulation to these areas.* In the introductory case, EM was given lidocaine without epinephrine to avoid tissue hypoxia in his digits.

### Distribution

Local anesthetics diverted into the systemic circulation travel in the venous system to the capillary bed of the lung. As the first capillary bed reached by the drug, the lung "cushions" the impact of the drug on the brain and other organs. The lung also metabolizes amide-linked LAs.

While traveling in the circulation, local anesthetics bind reversibly to two major plasma proteins: α-1 acid glycoprotein (an acute phase protein) and albumin. LAs can also bind to erythrocytes. Binding decreases as pH decreases, suggesting that the neutral form binds plasma proteins with higher affinity. Tissue binding occurs at the site of injection as well as other sites. The more hydrophobic the agent, the greater the tissue binding.

The volume of distribution ($V_d$) indicates the extent to which a drug distributes to the tissues from the systemic circulation. For the same amount of administered drug, a less hydrophobic LA (e.g., procaine) has a high plasma concentration (less is stored in tissues) and therefore a small $V_d$. A more hydrophobic LA (e.g., bupivacaine) has a low plasma concentration (more is stored in tissues) and, therefore, a large $V_d$. Local anesthetics with a larger $V_d$ are eliminated more slowly. (See Chapter 3, Pharmacokinetics, for a detailed discussion of the inverse relationship between $V_d$ and the elimination half-life of a drug.)

### Excretion

Ester-linked LAs are metabolized by tissue and plasma esterases (pseudocholinesterases). This process is fast (on the order of minutes), and the resulting products are excreted via the kidney. Amide-linked LAs are metabolized primarily in the liver by microsomal cytochrome P450 enzymes. The three major routes of metabolism are aromatic hydroxylation, N-dealkylation, and amide hydrolysis. Metabolites of amide-linked LAs are returned to the circulation and excreted by the kidney. Alterations in liver perfusion or in the maximum velocity of the liver enzymes can change the speed with which these agents are metabolized. LA metabolism is slowed in patients with cirrhosis or other liver diseases, and a standard dose of an amide-linked LA can lead to toxicity in such a patient. Some metabolism of amide-linked LAs can occur extrahepatically, for example, in the lung and kidney.

There are three phases in the distribution and excretion of local anesthetics following intravenous administration (Fig. 9-10). Rapid distribution delivers drug first to the highly perfused tissues (lung, brain, liver, heart, kidneys). This process rapidly lowers the plasma concentration of LA, and is largely completed within the first 5 minutes after injection. Next, during the alpha phase, there is slower redistribution of drug to the skeletal muscle and adipose tissue. This stage lasts for approximately 20–25 minutes. Finally, during the beta phase, the LA plasma concentration slowly decreases as drug (and metabolites) are excreted via the urine. This phase can last for up to 2 hours from the time of administration.

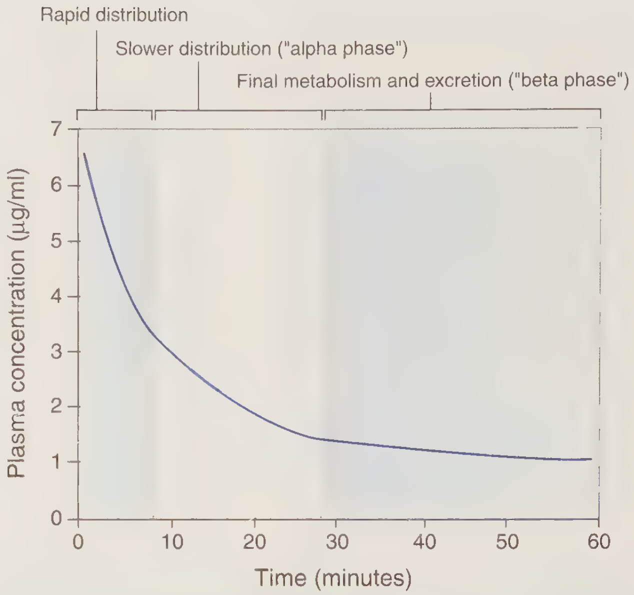


**Figure 9-10. Distribution and Elimination of Local Anesthetics.** Distribution and elimination of LAs occur in three phases, including a rapid phase of distribution to highly perfused tissues, a slower phase of distribution to moderately perfused tissues, and an even slower metabolism and excretion phase. In general, the time an LA spends in the circulation is relatively short. This time is especially short if the LA is very hydrophobic because, in that case, the LA distributes very rapidly out of the bloodstream into tissues.

## ADMINISTRATION OF LOCAL ANESTHETICS

Because the goal of local anesthesia is often to block transmission in a specific nerve, the method of administration can determine both the therapeutic effect and the extent of systemic toxicity. The following is an overview of the most common methods for administering local anesthetics.

### Topical Anesthesia

Topical anesthetics provide short-term pain relief when applied to mucous membranes or skin. The drug must cross the epidermal barrier, with the stratum corneum (outermost layer of epidermis) presenting the major obstacle, to reach the endings of Aδ-fibers and C-fibers in the dermis. Once across the epidermis, local anesthetics are absorbed rapidly into the circulation, increasing the risk of systemic toxicity. A mixture of tetracaine, adrenaline (epinephrine), and cocaine, known as **TAC,** is sometimes used before suturing small cuts. Because of concern about cocaine toxicity and/or addiction from this formulation, alternatives such as EMLA (see next page) are now used.

## Infiltration Anesthesia

Infiltration anesthesia is used to numb an area of skin via an injection. The local anesthetic is injected subcutaneously, often at several neighboring sites near the area to be anesthetized. This technique produces numbness much faster than does topical anesthesia, because the agent does not have to cross the epidermis. However, the injection can be painful because the solution, usually at an acidic pH, stings. Neutralization of the solution by addition of sodium bicarbonate can reduce most of the pain. The local anesthetics most commonly used for infiltration anesthesia are lidocaine, procaine, and bupivacaine.

## Peripheral Nerve Blockade

Peripheral nerve blockade can be subdivided into minor and major nerve blocks. For example, a minor nerve block for a distal extremity could involve the radial nerve, while a major nerve block for the entire arm would involve the brachial plexus. In both cases, local anesthetics are usually injected percutaneously. The amount injected is much more than would be necessary to block impulses in an unsheathed isolated nerve in vitro because the anesthetic must cross several layers of membranes before it reaches the target site (as discussed above), and most of the drug is removed by the local circulation. Therefore, only a small fraction of the injected drug actually reaches the nerve membrane. The choice of anesthetic usually depends on the desired duration of action. Epinephrine helps extend the duration of action of peripheral nerve blocks (but can also cause tissue hypoxia).

Brachial plexus blocks are particularly useful in the upper extremity because the entire arm can be anesthetized. Other useful peripheral blocks include intercostal blocks for the anterior abdominal wall, cervical plexus blocks for neck surgery, and sciatic and femoral blocks for the distal lower limb. In the introductory case, EM was given a digital nerve block, a type of peripheral block.

## Central Nerve Blockade

This type of blockade, where drug is delivered near the spinal cord, includes both epidural anesthesia and intrathecal (spinal) anesthesia. The early effects of these procedures result primarily from impulse blockade in spinal roots, but in later phases, anesthetic drug may act within the spinal cord. Bupivacaine is particularly useful as an epidural anesthetic during labor because, at low concentrations, it provides adequate pain relief without significant motor block. Reports of bupivacaine cardiotoxicity have led to decreased use of this agent in high concentrations (>0.5% weight/volume), although the dilute solutions used in obstetrics are rarely toxic. Newer, chemically similar drugs such as **ropivacaine** and **levobupivacaine** may be safer.

## Intravenous Regional Anesthesia

This type of local anesthesia is also called Bier's block. Both a tourniquet and a distally located elastic band are applied to an elevated extremity, leading to partial exsanguination of the limb. The tourniquet is then inflated and the band removed. The LA is then injected into a vein in the extremity to provide local anesthesia, and the tourniquet prevents systemic toxicity by limiting blood flow to and from the extremity. Intravenous regional anesthesia is occasionally used for arm and hand surgery.

# MAJOR TOXICITIES

Local anesthetics can have many potential toxicities, including effects on local tissues, peripheral vasculature, the heart, and the CNS. Hypersensitivity reactions are also possible. Administration of drug to a defined area usually limits the systemic side effects, but it is important to consider these potential toxicities whenever one is administering a local anesthetic.

Local anesthetics can cause local irritation. Skeletal muscle seems to be most sensitive to irritation with local anesthetic administration. Plasma levels of creatine kinase are elevated after intramuscular injection of LAs, indicating damage to muscle cells. This effect is usually reversible, and muscle regeneration is complete within a few weeks of the injection.

Local anesthetics have complex effects on the peripheral vasculature. Lidocaine, for example, initially causes vasoconstriction, but at longer times it produces vasodilation. Such biphasic actions may be attributable to separate effects on the vascular smooth muscle and on sympathetic nerves that innervate resistance arterioles. Bronchial smooth muscle is also affected in a biphasic manner. Initially, local anesthetics cause bronchoconstriction, but at later times, bronchorelaxation. The early event may reflect LA-induced release of calcium ions into the cytoplasm from intracellular stores, while the later effect may be caused by LA inhibition of plasma membrane sodium and calcium channels (see below).

The cardiac effect of LAs is primarily to reduce the conduction velocity of the cardiac action potential. Local anesthetics can act as antiarrhythmic drugs because of their ability to prevent ventricular tachycardia and ventricular fibrillation (an example of use-dependent block, see above). Lidocaine, for example, acts as both a local anesthetic and a class I antiarrhythmic (see Chapter 17). Local anesthetics also cause a dose-dependent decrease in cardiac contractility (a negative inotropic effect). The mechanism of this effect is not entirely understood, but may be caused by LA-mediated slow release of calcium from the sarcoplasmic reticulum, with a consequent reduction in the stores of calcium available to drive subsequent contractions. LAs can also directly inhibit calcium channels in the plasma membrane. The combination of reduced intracellular calcium storage and decreased calcium entry leads to decreased myocardial contractility.

Local anesthetics can have serious effects on the CNS. LAs are small amphipathic molecules that can rapidly cross the blood–brain barrier. Initially, LAs produce signs of CNS excitement, including tremors, tinnitus (ringing in the ears), shivering, twitching, and sometimes generalized convulsions. CNS excitation is followed by depression. It is hypothesized that, initially, local anesthetics selectively block inhibitory pathways in the cerebral cortex, resulting in the excitatory phase of CNS toxicity. As the concentration of LA increases in the CNS, all neuronal pathways—excitatory as well as inhibitory—are blocked, leading to CNS depression. Death can ultimately result from respiratory failure.

Hypersensitivity to local anesthetics is rare. This adverse effect is usually manifested as allergic dermatitis or asthma. LA-induced hypersensitivity occurs almost exclusively with ester-linked LAs. For example, a metabolite of procaine, para-aminobenzoic acid (PABA), is a known allergen.

## INDIVIDUAL AGENTS

Having discussed the general properties of local anesthetics, this section briefly presents the individual anesthetics in current clinical use, with an emphasis on the agents' differences in potency and half-life.

### Ester-Linked Local Anesthetics

#### *1. Procaine*

**Procaine** (*Novocain*) is a short-acting, ester-linked LA. Its low hydrophobicity allows for rapid removal of drug from the site of administration via the circulation, and results in little sequestration of drug in the local tissue surrounding the nerve. In the bloodstream, procaine is degraded rapidly by plasma pseudocholinesterases, and the metabolites are subsequently excreted in the urine. Its low hydrophobicity also causes procaine to dissociate rapidly from its binding site on the sodium channel, accounting for the low potency of this agent.

Procaine's primary use today is in infiltration anesthesia and in dental procedures. Occasionally it is used in diagnostic nerve blocks. Procaine is rarely used for peripheral nerve block because of its low potency, slow onset, and short duration of action. However, the rapidly hydrolyzed, and, therefore, short-acting homolog of procaine, **2-chloroprocaine** (Nesacaine), is popular as an obstetric anesthetic that is sometimes given epidurally just before delivery to control pain.

One of the metabolites of procaine is para-aminobenzoic acid (PABA), a compound required by some bacteria for purine and nucleic acid synthesis. The antibacterial sulfonamides are structural analogues of PABA that competitively inhibit the synthesis of an essential metabolite in folate biosynthesis (see Chapter 31, Pharmacology of Nucleotide Synthesis). Excess PABA can reduce the effectiveness of sulfonamides, and can, therefore, exacerbate bacterial infections. As mentioned above, PABA is also an allergen.

#### *2. Tetracaine*

**Tetracaine** is a long-acting, highly potent, ester-linked LA. Its long duration of action is caused by its high hydrophobicity—it has a butyl group attached to its aromatic group. This allows tetracaine to remain in the tissue surrounding a nerve for an extended period of time. Tetracaine's hydrophobicity also promotes prolonged interaction with its binding site on the sodium channel, accounting for its greater potency than lidocaine and procaine. It is mainly used in spinal and topical anesthesia. Its effective metabolism is slow, despite the potential for rapid hydrolysis by esterases because it is released only gradually from tissues into the bloodstream.

#### *3. Cocaine*

**Cocaine,** the prototypical and only naturally occurring LA, is ester-linked. It has a medium potency (one-half that of lidocaine) and a medium duration of action. Cocaine's structure is slightly unusual for local anesthetics. Its tertiary amine is part of a complex cyclic structure to which a secondary ester group is attached.

Cocaine's primary therapeutic uses today are in ophthalmic anesthesia and as part of the topical anesthetic TAC (tetracaine, adrenaline, cocaine; see above). Like prilocaine (see below), cocaine has a marked vasoconstrictive action that results from its inhibition of catecholamine uptake in synaptic terminals of both the peripheral and central nervous systems (see Chapter 8, Adrenergic Pharmacology). Inhibition of the uptake 1 system is also the mechanism for cocaine's profound cardiotoxic potential, and for the "high" associated with cocaine use. These euphoric properties limit the value of cocaine as a local anesthetic.

### Amide-Linked Local Anesthetics

#### *1. Lidocaine*

**Lidocaine**, the most commonly used LA and the one used in EM's case, is an amide-linked drug of moderate hydrophobicity. It has a rapid onset of action and a medium duration of action (about 1–2 hours) and is moderately potent. Lidocaine has two methyl groups on its aromatic ring, which enhance its hydrophobicity relative to procaine and slow its rate of hydrolysis.

Lidocaine has a relatively low pKa, and a large fraction of the drug is present in neutral form at physiologic pH. This results in rapid diffusion of the drug through membranes and a rapidly developing block. Lidocaine's duration of action is based on two factors: its moderate hydrophobicity and its amide linkage. The amide linkage prevents degradation of the drug by esterases, and its hydrophobicity allows the drug to remain near the area of administration (in local tissue) for a long time. The hydrophobicity also allows lidocaine to bind more tightly than procaine to the LA binding site on the sodium channel, enhancing its potency. The vasoconstrictive effects of coadministered epinephrine can extend lidocaine's duration of action significantly.

Lidocaine is used in infiltration, peripheral nerve block, epidural, spinal, and topical anesthesia. It is also used as a Class I antiarrhythmic. The mechanism of its antiarrhythmic action is its blocking of sodium channels in cardiac myocytes. Lidocaine's slow metabolism in the circulation makes it a useful antiarrhythmic (see Chapter 17). More potent amide-linked LAs, such as bupivacaine, bind too tightly to cardiac sodium channels to serve as useful antiarrhythmics; such drugs cause either conduction blocks or tachyarrhythmias.

Lidocaine undergoes metabolism in the liver, where it is first dealkylated by mixed function oxidases. Subsequently, it undergoes hydrolysis and hydroxylation. Lidocaine's metabolites have only weak anesthetic activity.

The toxicities of lidocaine are manifested mainly in the CNS and heart. Adverse effects can include drowsiness, tinnitus, twitching, and even seizures. CNS depression and cardiotoxicity can occur at high plasma levels of the drug.

**Prilocaine** is similar to lidocaine, except that it has vasoconstrictive activity as well as local anesthetic activity. Because prilocaine does not require concurrent administration of epinephrine to prolong its duration of action, this drug is a good choice for patients in whom epinephrine is contraindicated.

### 2. Bupivacaine

**Bupivacaine** is an amide-linked LA with a long duration of action. It is highly hydrophobic (and, therefore, highly potent) as a result of its butyl group attached to the tertiary nitrogen. Dilute bupivacaine applied epidurally has more effect on nociception than on locomotor activity. This property, combined with the drug's long duration of action and high potency, has made bupivacaine useful in spinal, epidural, and peripheral nerve block, and in infiltration anesthesia. Bupivacaine is metabolized in the liver, where it undergoes N-dealkylation by cytochrome P450 enzymes. It has been used widely in low concentrations for labor and post-operative anesthesia, because it provides about 2–3 hours of pain relief without immobilizing motor blockade. However, because of its cardiotoxicity at higher concentrations, bupivacaine is no longer used as commonly for these purposes. (The drug blocks cardiac myocyte sodium channels during systole, but is very slow to dissociate during diastole. Thus, it can trigger arrhythmias through the generation of re-entry pathways.)

Because bupivacaine contains a chiral center, it exists as a racemic mixture of mirror-image R-enantiomers and S-enantiomers. The R-enantiomers and S-enantiomers are now known to have different affinities for the sodium channel and, therefore, different cardiovascular effects. The S-enantiomer has been separated and marketed as the safer and less toxic **levobupivacaine,** as has its structurally homologous relative **ropivacaine.**

### 3. EMLA

**EMLA** (Eutectic Mixture of Local Anesthetic) is a combination of lidocaine and prilocaine that is delivered as a topically applied cream or patch. EMLA is useful clinically because it has a higher concentration of local anesthetic per drop contacting the skin than standard topical preparations. It is effective in a number of situations including venipuncture, arterial cannulation, lumbar puncture, and dental procedures, particularly in children who dread the pain of injections.

## Conclusion and Future Directions

Local anesthetics are vital to the practice of medicine and surgery because they can block pain sensations regionally. Their clinical actions involve blocking pain neurons called nociceptors. Nociceptors are afferent neurons whose small axons are classified as either Aδ- or C-fibers. Local anesthetics block all types of nerve fibers running in peripheral nerves, including those of nociceptors, by blocking voltage-gated sodium channels in neuronal membranes. LAs act on sodium channels from the cytoplasmic side of the membrane.

In general, local anesthetics have an aromatic group that is connected to an ionizable tertiary amine via an ester or amide linkage. This structure is common to almost all local anesthetics and contributes to their function. Both the hydrophobicity, attributable in large part to the aromatic ring and its substituents, and the ionizability (pKa) of the tertiary amine determine the potency of the LA and the kinetics of local anesthetic action. Molecules with pKa values of 8–10 (weak bases) are the most effective as local anesthetics. The neutral form can cross membranes to reach the LA binding site on the sodium channel, and the protonated form is available to bind with high affinity to the target site.

The sodium channel exists in three forms: open, inactivated and resting. There are also several transient "closed" forms between the resting and open states. Local anesthetics bind strongly to the closed, open, and inactivated conformations of the sodium channel. This tight binding inhibits the return of the channel to the resting state, extends the refractory period, and inhibits the transmission of action potentials.

Local anesthetics seem to have effects beyond their inhibition of sodium channels in nerve fibers. Some of these ancillary effects show therapeutic promise and could potentially lead to other indications for LAs. For example, LAs have been reported to affect wound healing, thrombosis, hypoxia/ischemia-induced brain injury, and bronchial hyperactivity. LAs are also being investigated for use in chronic and neuropathic pain management, such as that seen in patients with diabetic neuropathy, post-herpetic neuralgia, burns, cancer, and strokes. The development of ultralong-acting LAs (whose effects could last for days) is continuing to be investigated; these studies involve altering LA structure at the molecular level, using a variety of drug delivery systems, and discovering different classes of neuronal impulse blockers.

One exciting area of research is the potential effect of LAs on the inflammatory response. LAs have been found to inhibit the recruitment and function of polymorphonuclear granulocytes (PMNs). Because PMNs do not express sodium channels, the mechanism of inhibition likely differs from the mechanism of LA-induced anesthesia. Preliminary studies have found that LAs affect several steps in inflammation including cytokine release, PMN migration to the site of injury, PMN accumulation at the site, and free radical release at the site. Applications to diseases in which the inflammatory response is overactive, such as acute respiratory distress syndrome (ARDS), inflammatory bowel disease, and myocardial infarction and reperfusion injury are being investigated.

## Suggested Readings

Butterworth JF, Strichartz, GR. 1990. Molecular mechanisms of local anesthesia: A review. Anesthesiology 1990;72:711–734. (*A detailed and complex review for those who want to delve deeper.*)

Cousins MJ, Bridenbaugh PO. Neural Blockade. 3rd Ed. New York: Lippincott-Raven, 1998. (*A detailed but readable textbook. A good follow-up reference to concepts discussed in this chapter.*)

Smith C. Pharmacology of local anesthetic agents. Br J Hosp Med 1994;52:455–460. (*Includes extensive information on pharmacokinetics.*)

## Drug Summary Table Local Anesthetic Pharmacology

| Drug | Clinical Applications | Serious and Common Adverse Effects | Contraindications | Therapeutic Considerations |
|---|---|---|---|---|
| **ESTER-LINKED LOCAL ANESTHETICS**<br>*Mechanism—Inhibit voltage-gated sodium channels in excitable cell membranes* | | | | |
| **Procaine** | Infiltration anesthesia, diagnostic nerve blocks | | Sulfonamides | Short-acting, low potency |
| **2-Chloroprocaine** | Obstetric epidural anesthesia | | Opiate antagonist, myasthenia gravis (MG) | Even more short-acting analogue of procaine |
| **Tetracaine** | Spinal, topical anesthesia | | Sulfonamides, MG, liver disease | |
| **Cocaine** | Ophthalmic anesthesia | Cardiovascular toxicity, addictive potential | Pregnancy | Has intrinsic vasoconstrictive activity |
| **Tetracaine, adrenaline (epinephrine), cocaine (TAC)** | Ophthalmic anesthesia | | | Combination formulation |
| **AMIDE-LINKED LOCAL ANESTHETICS**<br>*Mechanism—Same as above* | | | | |
| **Lidocaine** | Widely used local anesthetic, antiarrhythmic | | Severe atrioventricular, intraventricular block unless have pacemaker | |
| **Prilocaine** | Dental procedures, nerve block | | | Has intrinsic vasoconstrictive activity |
| **Bupivacaine** | Obstetric epidural anesthesia | Arrhythmias, inhibits myocyte contractility | | Sensory block without much motor block, low concentrations for epidural anesthesia |
| **EMLA (eutectic mixture of lidocaine and prilocaine)** | Topical—venipuncture, lumbar puncture, dental procedures, arterial blood gas | | | Combination formulation |
| **Ropivacaine** | Obstetric epidural anesthesia | Less cardiotoxic than bupivacaine | | S-enantiomer only, sensory > motor blockade |
| **Levobupivacaine** | Peripheral, epidural | Less cardiotoxic than bupivacaine | | |

# IIc

# Principles of Central Nervous System Pharmacology

# 10

# Pharmacology of Excitatory and Inhibitory Neurotransmission

Janet Chou, Gary R. Strichartz, and Eng H. Lo

**Introduction**
**Case**
**Amino Acids as Excitatory and Inhibitory CNS Neurotransmitters**
**Physiology of the Glutamate Receptor**
  Glutamate Synthesis and Degradation
  Ionotropic Glutamate Receptors
  Metabotropic Glutamate Receptors
**Pathophysiology and Pharmacology of Glutamatergic Neurotransmission**
  Stroke and Trauma
  Hyperalgesia
  Epilepsy
  Neurodegenerative Diseases
**Physiology of the GABA Receptor**
  GABA Synthesis and Degradation
  GABA Receptors
    *$GABA_A$ Receptor*
    *$GABA_B$ Receptor*
    *$GABA_C$ Receptor*
**Pharmacologic Classes and Agents Affecting GABAergic Neurotransmission**
  $GABA_A$ Receptor Agonists and Antagonists
  Barbiturates
    *Mechanism of Action*
    *Clinical Applications*
    *Pharmacokinetics and Metabolism*
    *Toxicity*
    *Tolerance and Dependence*
  Benzodiazepines
    *Mechanism of Action*
    *Clinical Applications*
    *Pharmacokinetics and Metabolism*
    *Toxicity*
    *Tolerance and Dependence*
  Nonprescription Uses of Drugs that Alter GABA Physiology
    *Ethanol*
    *Chloral Hydrate and Flunitrazepam*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Excitatory and inhibitory neurotransmitters regulate a diverse array of behavioral processes including sleep, learning, memory, and pain sensation. Excitatory and inhibitory neurotransmitters are also implicated in such pathologic processes as epilepsy and neurotoxicity. The interactions among ion channels, the receptors that regulate these channels, and amino acid neurotransmitters in the central nervous system (CNS) constitute the molecular basis for these processes. Many of the drugs that manipulate excitatory and inhibitory activity in the CNS alter amino acid neurotransmission, most commonly by modulating a receptor that controls one of these ion channels. This chapter discusses the physiology, pathophysiology, and pharmacology of the two most important amino acid neurotransmission systems in the CNS, those involving **glutamate** and **γ-aminobutyric acid (GABA).**

## Case

S.B., a 70-year-old man, is having trouble sleeping. He recalls that his sister has been prescribed phenobarbital, a barbiturate, to control her epileptic seizures, and that barbiturates are sometimes also prescribed as sleeping pills. He decides to take "just a few" with some alcohol to help him sleep. Shortly afterward, S.B. is rushed to the emergency room after his sister finds him passed out next to the open bottles of alcohol and barbiturates. He is difficult to arouse and dysarthric, with an unsteady gait and impaired attention and memory. His respiratory rate has decreased to approximately six shallow breaths per minute. The emergency department team inserts an endotracheal tube to prevent him from aspirating gastric contents. Activated charcoal is administered through a nasogastric tube to prevent further absorption of phenobarbital into his bloodstream. S.B. also receives intravenous sodium bicarbonate to alkalinize his urine (final pH = 7.5) and thus facilitate drug excretion. Three days later, he has recovered sufficiently to return home.

## QUESTIONS

- 1. How do barbiturates act to control epileptic seizures and to induce sleep?
- 2. How does the patient's age affect the extent of CNS depression caused by barbiturates?
- 3. What is the role of ethanol in CNS depression? How does ethanol interact with barbiturates?
- 4. What are the signs of barbiturate poisoning, and how are these signs explained by the drug's mechanism of action?

## AMINO ACIDS AS EXCITATORY AND INHIBITORY CNS NEUROTRANSMITTERS

The CNS has uniquely high concentrations of certain amino acids that bind to postsynaptic receptors and, thereby, act as excitatory or inhibitory neurotransmitters. Of the two main classes of neuroactive amino acids, **glutamate** is the primary excitatory amino acid; whereas, **γ-aminobutyric acid (GABA)** is the major inhibitory amino acid.

Amino acid neurotransmitters ultimately elicit excitatory or inhibitory responses by altering the conductance of one or more ion-selective channels. An excitatory neurotransmitter could open a cation-specific channel, such as a sodium channel, and thereby cause a net influx of sodium ions that depolarizes the membrane. Alternatively, an excitatory (depolarizing) response could result if a neurotransmitter closes potassium "leak channels" and, thereby, reduces the outward flow of potassium ions that occurs, even at rest (see Chapter 5, Principles of Cellular Excitability and Electrochemical Transmission). Notice that, in both of these examples, excitatory amino acid neurotransmitters cause a net inward current.

In contrast, inhibitory neurotransmitters trigger a selective outward current. For example, inhibitory neurotransmitters could open $K^+$ channels or $Cl^-$ channels and, thereby, induce $K^+$ efflux or $Cl^-$ influx, respectively. Either type of ion movement—the loss of intracellular cations or the gain of intracellular anions—results in membrane hyperpolarization and decreased membrane resistance (Fig. 10-1). Therefore, the excitatory or inhibitory effect elicited by a neurotransmitter is a consequence of the changes it causes in the target cell's ion fluxes.

A neurotransmitter can generate responses of varying magnitude depending on its local concentration and the number of its presynaptic and postsynaptic receptor sites. The extent of the transmitter's action on other receptors and ion conductances can also contribute to the size of the cellular response. Notably, the concentration of agonist (either exogenous, such as a drug, or endogenous, such as a neurotransmitter) often has a long-term influence on receptor expression. A persistent excess of agonist can decrease receptor number or receptor signaling via desensitization, which occurs in fractions of seconds, or down-regulation, which occurs over hours; both processes induce tolerance to the neurotransmitter. Conversely, a persistent deficit of transmitter can increase receptor expression and cause target cell hypersensitivity. Chapter 16, Pharmacology of Chemical Dependence and Addiction, discusses the mechanisms of these effects in detail. The treatment of chronic CNS disorders must consider the consequences of these adaptations because the effects of a drug may evolve during the course of an extended therapeutic regimen.

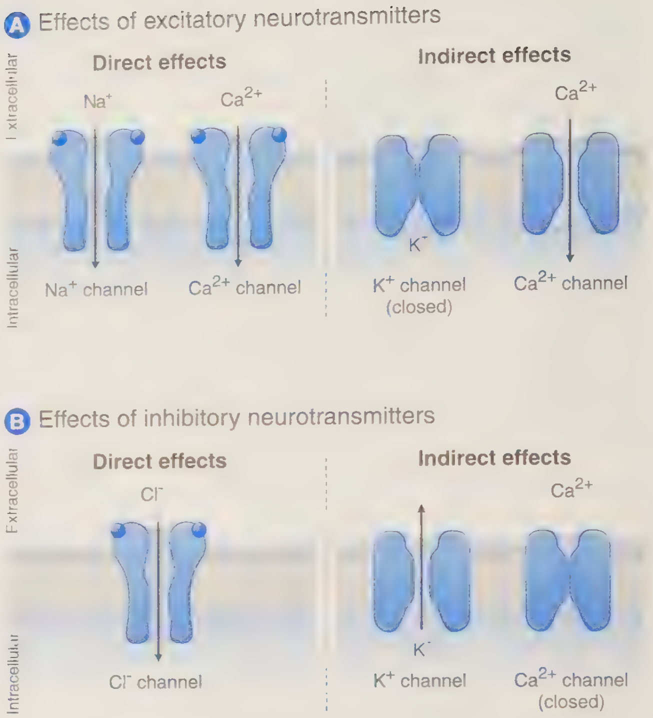


**Figure 10-1. Effects of Excitatory and Inhibitory Neurotransmitters on Ion Conductances.** **A.** Excitatory neurotransmitters depolarize membranes by inducing a net inward current, either by enhancing inward current (for example, opening a $Na^+$ channel) or by reducing outward current (for example, closing a $K^+$ channel). Potassium channel closure also increases the resting membrane resistance and renders the cell more responsive to excitatory postsynaptic currents. The increased membrane resistance results in greater responsiveness (i.e., a greater change in $V_m$ per change in current) because $\Delta V_m = \Delta i_m \cdot r_m$, where $V_m$ is the membrane potential, $i_m$ is the excitatory current, and $r_m$ is the membrane resistance. **B.** Inhibitory neurotransmitters hyperpolarize membranes by inducing a net outward current, by promoting either an influx of anions (for example, opening a $Cl^-$ channel) or an efflux of cations (for example, opening a $K^+$ channel). Opening of chloride or potassium channels also lowers the membrane resistance and, thereby, lowers the $\Delta V_m$ response to excitatory currents.

## PHYSIOLOGY OF THE GLUTAMATE RECEPTOR

Glutamatergic synapses exist throughout the CNS. There are two main classes of glutamate receptors: ligand-gated ion channels—termed **ionotropic** receptors—and G protein-coupled **metabotropic** receptors (see Chapter 1, Drug–Receptor Interactions). The binding of glutamate to its receptors initiates a set of molecular and cellular events associated with neuronal excitation, as detailed below. Glutamate receptors are also important in the development of elevated pain sensation (hyperalgesia), in cerebral neurotoxicity, and in the synaptic changes involved in certain types of memory formation (see Chapter 15, Pharmacology of Analgesia).

## GLUTAMATE SYNTHESIS AND DEGRADATION

Glutamate synthesis occurs via two distinct pathways. In one pathway, $\alpha$-ketoglutarate formed in the Krebs cycle is transaminated to glutamate in CNS nerve terminals (Fig. 10-2A). Alternatively, glutamine produced by glial cells is transported into nerve terminals and converted to glutamate by the enzyme glutaminase (Fig. 10-2B).

Glutamate, as with most other neurotransmitters, is released through calcium-dependent exocytosis of transmitter-containing vesicles. Glutamate is removed from the synaptic cleft by presynaptic (neuronal) and glial cell glutamate reuptake transporters. These transporters are $Na^+$ dependent and have a high affinity for glutamate. In glial cells, the enzyme glutamine synthetase converts glutamate into glutamine, which is recycled into adjacent nerve terminals for conversion back to glutamate. Glutamine generated in glial cells can also enter the Krebs cycle and undergo oxidation; the resulting $\alpha$-ketoglutarate enters neurons to replenish the $\alpha$-ketoglutarate consumed during glutamate synthesis (Fig. 10-2B).

## IONOTROPIC GLUTAMATE RECEPTORS

Ionotropic glutamate receptors mediate fast excitatory synaptic responses. These receptors are multisubunit cation channels that, upon activation, permit the flow of $Na^+$, $K^+$, and, in some instances, $Ca^{2+}$ ions across membranes. Ionotropic glutamate receptors are thought to be either tetramers or pentamers, although conclusive evidence of the number of subunits in the complex is not yet available (Fig. 10-3A). There are three main subtypes of glutamate-gated ion channels, classified according to their selective activation by the agonists **AMPA, kainate,** and **NMDA.** The diversity of functionally distinct ionotropic receptors arises from differences in protein sequence because of alternative mRNA splicing and post-transcriptional mRNA editing, and from the use of different combinations of subunits to form the receptors.

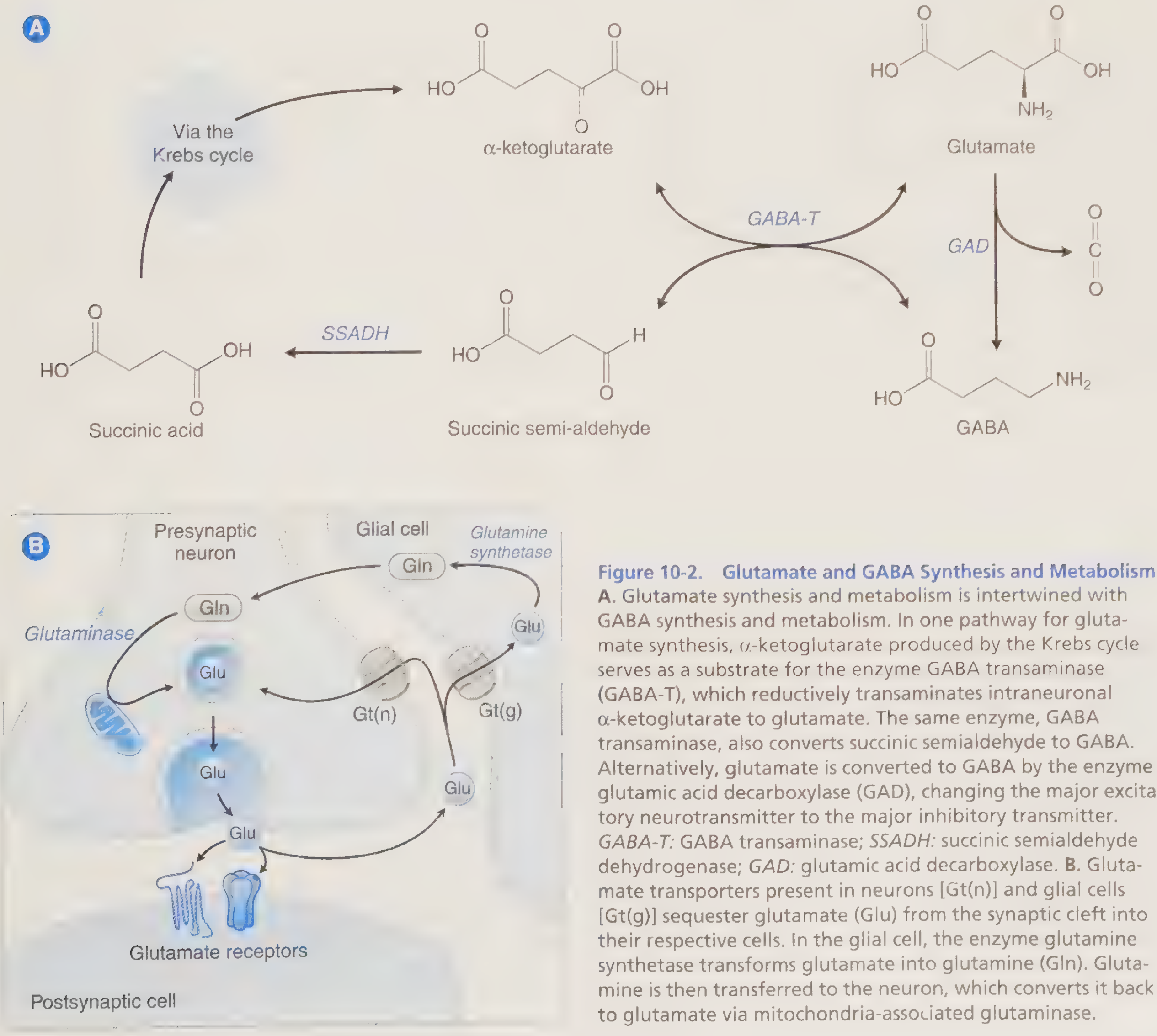


**Figure 10-2. Glutamate and GABA Synthesis and Metabolism.** **A.** Glutamate synthesis and metabolism is intertwined with GABA synthesis and metabolism. In one pathway for glutamate synthesis, $\alpha$-ketoglutarate produced by the Krebs cycle serves as a substrate for the enzyme GABA transaminase (GABA-T), which reductively transaminates intraneuronal $\alpha$-ketoglutarate to glutamate. The same enzyme, GABA transaminase, also converts succinic semialdehyde to GABA. Alternatively, glutamate is converted to GABA by the enzyme glutamic acid decarboxylase (GAD), changing the major excitatory neurotransmitter to the major inhibitory transmitter. *GABA-T:* GABA transaminase; *SSADH:* succinic semialdehyde dehydrogenase; *GAD:* glutamic acid decarboxylase. **B.** Glutamate transporters present in neurons [Gt(n)] and glial cells [Gt(g)] sequester glutamate (Glu) from the synaptic cleft into their respective cells. In the glial cell, the enzyme glutamine synthetase transforms glutamate into glutamine (Gln). Glutamine is then transferred to the neuron, which converts it back to glutamate via mitochondria-associated glutaminase.

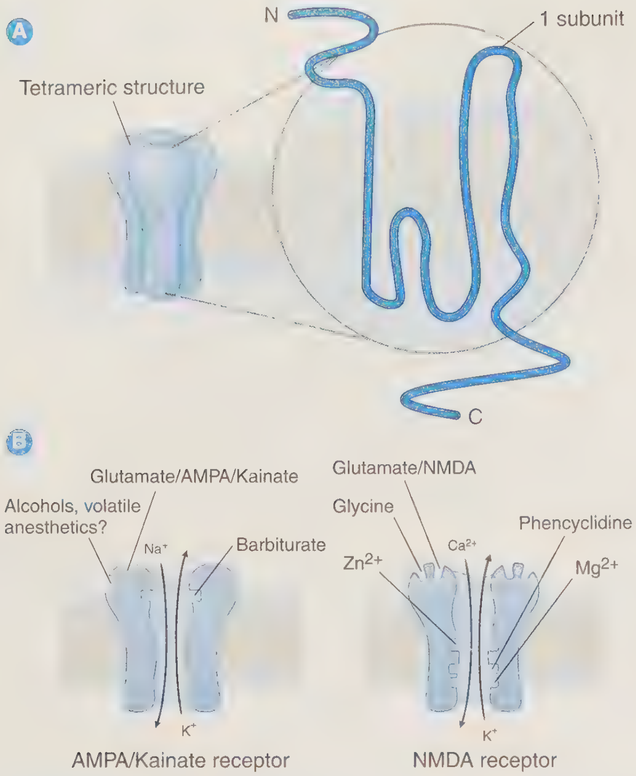


**Figure 10-3. Schematic Representation of the Ionotropic Glutamate Receptors.** **A.** Although ionotropic glutamate receptors were originally thought to be pentamers, more recent studies suggest that they may instead be composed of only four subunits (left). The structure on the right shows one ionotropic glutamate receptor subunit, which seems to span the membrane three times. **B.** Major binding sites on the AMPA/kainate and NMDA classes of ionotropic glutamate receptors are shown. Although there is indirect evidence for the location of many of the drug binding sites that are schematically indicated in this diagram, the definitive localization of these sites remains to be determined.

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid) receptors are located throughout the CNS, particularly in the hippocampus and cerebral cortex. Four AMPA receptor subunits (GluR1–GluR4) have been identified (Table 10-1). AMPA receptor activation results primarily in $Na^+$ influx (as well as some $K^+$ efflux), allowing these receptors to regulate fast, excitatory postsynaptic depolarization at glutamatergic synapses (Fig. 10-3B). Although most AMPA receptors in the CNS have low $Ca^{2+}$ permeability, the absence of certain subunits (such as GluR2) from the receptor complex increases the $Ca^{2+}$ permeability of the channel. Calcium entry through AMPA receptors may play a role in neuronal damage. For example, an animal model of status epilepticus demonstrates that increased expression of $Ca^{2+}$-permeable glutamate receptors precedes hippocampal neurodegeneration. As discussed below, excessive levels of intracellular calcium can initiate molecular events that culminate in neuronal death.

Kainate receptors are found throughout the CNS, particularly in certain areas of the hippocampus and cerebellum. Five kainate receptor subunits have been identified (Table 10-1). Like AMPA receptors, kainate receptors permit $Na^+$ influx and $K^+$ efflux through channels that possess rapid activation and deactivation kinetics. Also, as with AMPA receptors, the combination of subunits in the kainate receptor complex determines the channel's $Ca^{2+}$ permeability. Experiments using kainate receptor-selective agents have allowed the assignment of specific receptor functions. For example, in the spinal cord, kainate receptors participate in pain transmission. At the presynaptic (nociceptor) terminal, activation of kainate receptors reduces glutamate release from the terminal. Postsynaptic kainate receptors may contribute to the coding of pain intensity.

NMDA (N-methyl-D aspartate) receptors are located throughout the CNS, most notably in the hippocampus, cerebral cortex, and spinal cord. These receptors consist of multisubunit oligomeric transmembrane complexes. NMDA receptor activation, which requires simultaneous binding of the neurotransmitter glutamate and the cofactor glycine, opens a channel that allows $K^+$ efflux as well as $Na^+$ and $Ca^{2+}$ influx (Fig. 10-3B). NMDA receptors are often co-localized with AMPA or kainate receptors at glutamatergic synapses. Importantly, while AMPA and kainate receptor activation results in channel opening at resting membrane potentials, $Mg^{2+}$ ions block the channel pore of even glutamate- and glycine-bound NMDA receptors in the resting membrane. Concurrent depolarization of the membrane is required to relieve this voltage-dependent $Mg^{2+}$ block. Either trains of postsynaptic action potentials or activation of AMPA/kainate receptors in adjacent regions of the membrane can cause the postsynaptic membrane depolarization that is necessary to unblock the NMDA receptor. Therefore, NMDA receptors differ from the other ionotropic glutamate receptors because of their multiple requirements for channel activation: to open the receptor channel, the conformational gating that results from the dual binding of glycine and glutamate to the receptor must be accompanied by membrane depolarization that removes the $Mg^{2+}$ block of the channel pore (Fig. 10-3B).

## METABOTROPIC GLUTAMATE RECEPTORS

Metabotropic glutamate receptors (mGluR) consist of a seven transmembrane–spanning receptor coupled via GTP-binding proteins (G proteins) to various effector mechanisms (Fig. 10-4). At least eight subtypes of metabotropic glutamate receptors exist; each belongs to one of three subgroups (groups I, II, and III) according to its sequence homology, signal transduction mechanisms, and pharmacology.

Subgroup I receptors cause neuronal excitation either through phospholipase C (PLC) activation and subsequent $IP_3$-mediated intracellular $Ca^{2+}$ release, or through adenylyl cyclase activation and cAMP generation. In contrast, subgroup II and III receptors inhibit adenylyl cyclase and decrease cAMP synthesis (Table 10-2). These second messenger systems enable metabotropic glutamate receptors to alter ion fluxes. For example, phosphorylation of ion channels by protein kinases, several of which are activated by elevated intracellular $Ca^{2+}$ and by PLC-generated diacylglycerol, can regulate channel opening and closing rates and, thus, determine membrane ion permeabilities. These changes give rise to the postsynaptic excitatory effects associated with mGluR activation, such as increased neuronal firing. In the hippocampus, neocortex, and cerebellum, metabotropic glutamate re-

**TABLE 10-1** Classification of Ionotropic Glutamate Receptor Subtypes

| IONOTROPIC GLUTAMATE RECEPTOR SUBTYPE | SUBUNITS | AGONISTS | ACTIONS |
|---|---|---|---|
| AMPA | GluR1<br>GluR2<br>GluR3<br>GluR4 | Glutamate or AMPA | Increase $Na^+$ and $Ca^{2+}$ influx, increase $K^+$ efflux (N.B. receptors with GluR2 have ion channels with decreased $Ca^{2+}$ permeability) |
| Kainate | GluR5<br>GluR6<br>GluR7<br>KA1<br>KA2 | Glutamate or kainate | Increase $Na^+$ influx, increase $K^+$ efflux |
| NMDA | NR1<br>NR2A<br>NR2B<br>NR2C<br>NR2D | Glutamate or NMDA and glycine and membrane depolarization | Increase $Ca^{2+}$ influx, increase $K^+$ efflux |

ceptor activation increases neuronal firing rates by inhibiting a hyperpolarizing $K^+$ current. Subgroup I mGluRs can also elicit an excitatory response by inhibiting $GABA_A$ receptor-mediated chloride currents (discussed below) and by facilitating ionotropic glutamate receptor-mediated cation currents. Presynaptic mGluRs, such as subgroup II and III receptors in the hippocampus, can operate as inhibitory autoreceptors that inhibit presynaptic $Ca^{2+}$ channels and thereby limit the release of glutamate when synaptic concentrations rise too high or last too long. Thus, the diversity of metabotropic glutamate receptor subtypes and signaling mechanisms enables these receptors to serve a range of functions, including both presynaptic inhibitory effects and postsynaptic excitatory effects.

## PATHOPHYSIOLOGY AND PHARMACOLOGY OF GLUTAMATERGIC NEUROTRANSMISSION

**Excitotoxicity** refers to neuronal death caused by excessive cellular excitation caused by toxic levels of glutamatergic neurotransmission. Overly high levels of synaptic glutamate, abnormal upregulation of glutamate receptors, or heightened glutamate sensitivity can lead to this condition. Normally, termination of glutamate receptor activation occurs via transmitter reuptake by presynaptic and glial transporters, transmitter

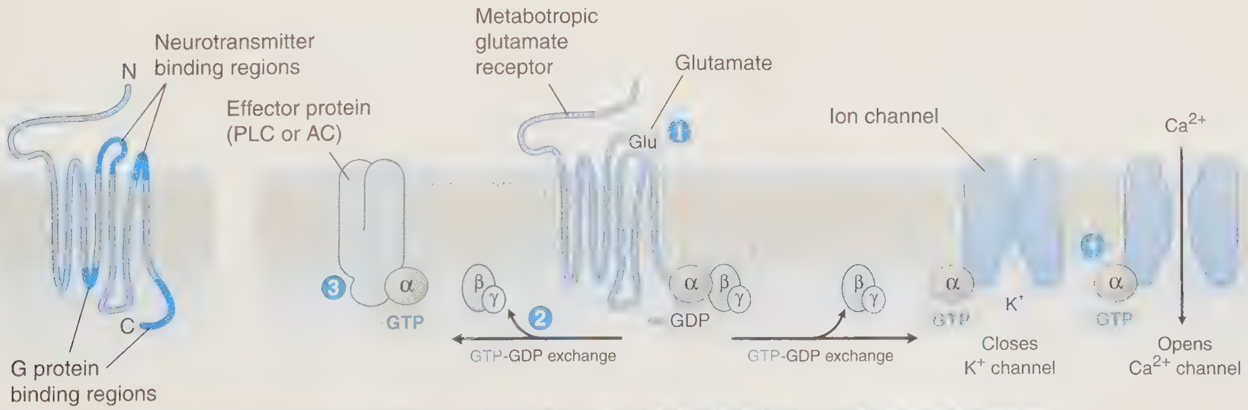


Figure 10-4. Schematic Representation and Downstream Signaling of the Metabotropic Glutamate Receptor Metabotropic glutamate receptors are seven transmembrane-spanning proteins with an extracellular ligand binding site and an intracellular G protein binding site (left). Ligand binding to the metabotropic glutamate receptor results in GTP association with the α-subunit of the G protein (1; right). The GTP-associated α subunit then dissociates from the βγ dimer (2). $G_\alpha$ and $G_{\beta\gamma}$ can then activate effector proteins such as adenylyl cyclase (AC) and phospholipase C (PLC; 3). G proteins can also open or close ion channels directly (4).

**TABLE 10-2** Metabotropic Glutamate Receptor (mGluR) Subtypes and Their Actions

| GROUP | SUBTYPE | ACTIONS |
|---|---|---|
| I | mGluR1<br>mGluR5 | Activates adenylyl cyclase → increases cAMP (mGluR1 only)<br>Increases PLC activity → PIP$_2$ hydrolysis → increases IP$_3$ and DAG → increases Ca$^{2+}$ levels, stimulates PKC<br>Inhibits K$^+$ channels |
| II | mGluR2<br>mGluR3 | Inhibits adenylyl cyclase → decreases cAMP<br>Inhibits voltage-sensitive Ca$^{2+}$ channels<br>Activates K$^+$ channels |
| III | mGluR4<br>mGluR6<br>mGluR7<br>mGluR8 | Inhibits adenylyl cyclase → decreases cAMP<br>Inhibits voltage-sensitive Ca$^{2+}$ channels |

Group I mGluRs activate adenylyl cyclase and phospholipase C (PLC), while group II and group III mGluRs inhibit adenylyl cyclase. The downstream effects of mGluRs on ion channels are complex and varied. Some of the main actions on ion channels are listed. Note that the actions of group I receptors are generally excitatory, while those of groups II and III receptors are generally inhibitory.

diffusion out of the synaptic cleft, or receptor desensitization. As described below, however, increased release or decreased reuptake of glutamate in pathologic states can lead to a positive feedback cycle involving increased intracellular Ca$^{2+}$ levels, cellular damage, and further glutamate release. Some of the disease states associated with excitotoxicity include CNS ischemia, physical trauma, epilepsy, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease. Although no genetic diseases have been correlated with glutamate receptor mutations in humans, experimental mutations in animals have suggested a role for glutamate receptors in the pathologic conditions discussed below.

The development of therapeutics directed at glutamatergic neurotransmission remains in a preliminary stage; indeed, the difficulties encountered in clinical trials of these drugs have revealed the complexities of selectively modulating glutamate receptor function. Noncompetitive receptor antagonists, such as drugs that block the channel's pore, have a theoretical advantage over competitive antagonists that act at the agonist binding site because the latter could be displaced by glutamate at the high levels present during ischemia or seizures. Both types of glutamate receptor antagonists could disrupt normal synaptic transmission, leading to undesirable side effects or toxicity from the overinhibition of excitatory neurotransmission. In humans, the side effects associated with overinhibition of glutamatergic neurotransmission include respiratory depression, blood pressure changes, sedation, ataxia, dysphoria, encephalopathy, paranoia, hallucinations, catatonia, and memory loss.

**Memantine** is one example of a noncompetitive NMDA receptor antagonist that does not cause the side effects associated with competitive receptor antagonists. The action of the drug is "use-dependent;" it becomes an effective antagonist only after repeated receptor activation and channel opening. As such, memantine allows low levels of receptor activation during physiologic stimulation, but prevents the higher levels of activation that could occur in such pathologic states as hypoxia. (See Chapters 9 and 17 for a more extended discussion of use-dependent channel blockers.) Clinical trials have suggested that the neuroprotective effects of this drug, attributed to its reversible blockade of the open, Mg$^{2+}$-free channel pore, may effectively limit the dementia in Alzheimer's disease and minimize the neurodegeneration associated with trauma, ALS, Parkinson's disease, and epilepsy.

The diversity of glutamate receptors and receptor subunits constitutes a potential advantage for the development of glutamate receptor antagonists that are selective for a particular receptor subtype. In the future, highly specific antagonists for glutamate receptor subtypes could potentially protect the CNS in stroke, prevent hyperalgesia after tissue trauma, and treat epileptic seizures.

## STROKE AND TRAUMA

Stroke and trauma can reduce CNS blood flow to levels that cause ischemia and tissue damage. Low oxygen levels diminish oxidative metabolism and cellular ATP production, which impairs the maintenance of transmembrane ion gradients. The flow of ions down their electrochemical gradients causes membrane depolarization and elevation of intracellular Na$^+$ and Ca$^{2+}$ concentrations. Dissipation of ion gradients also inhibits the action of Na$^+$-coupled glutamate transporters that remove extracellular glutamate, thus prolonging the effects of glutamate in the synaptic cleft. Rising glutamate levels activate Ca$^{2+}$-permeable NMDA receptor-coupled channels. The resultant accumulation of intracellular Ca$^{2+}$ causes additional glutamate release and activates many Ca$^{2+}$-dependent degradation enzymes (DNAses, proteases, phosphatases, phospholipases), and nitric oxide synthase. The influx of extracellular Ca$^{2+}$ through depolarized membranes also injures mitochondria, overwhelming their high storage capacity for $Ca^{2+}$. Damaged mitochondria release pro-apoptotic factors and lose the ability to quench free radicals. In summary, glutamate-mediated excitotoxicity consists of a positive feedback loop involving excessive $Ca^{2+}$ influx, increased glutamate release, and cellular damage that is difficult to reverse once it reaches a critical level (Fig. 10-5).

Shortly after its discovery, the highly $Ca^{2+}$-permeable NMDA receptor was viewed as a major contributor to neuronal cell death caused by $Ca^{2+}$ overload. Consistent with this, NMDA receptor antagonists were shown to decrease neuronal injury resulting from excessive NMDA receptor activation, particularly in animal models subjected to repeated episodes of partial or brief ischemia. Clinical trials of such drugs in stroke patients have not been successful, however. The failure of these drugs to decrease stroke-induced neuronal death may stem from differences between animal models and human pathophysiology. For example, animal models may not account for the broad heterogeneity of stroke subtypes in humans. In addition, the concentrations of drug necessary to exert significant neuroprotective effects seem to block normally functioning glutamate receptors in healthy brain tissue, leading to schizophrenia-like effects, memory impairment, and neurotoxic reactions. Future pharmacologic research may promote the development of drugs with fewer side effects, such as the noncompetitive NMDA receptor antagonist memantine mentioned above.

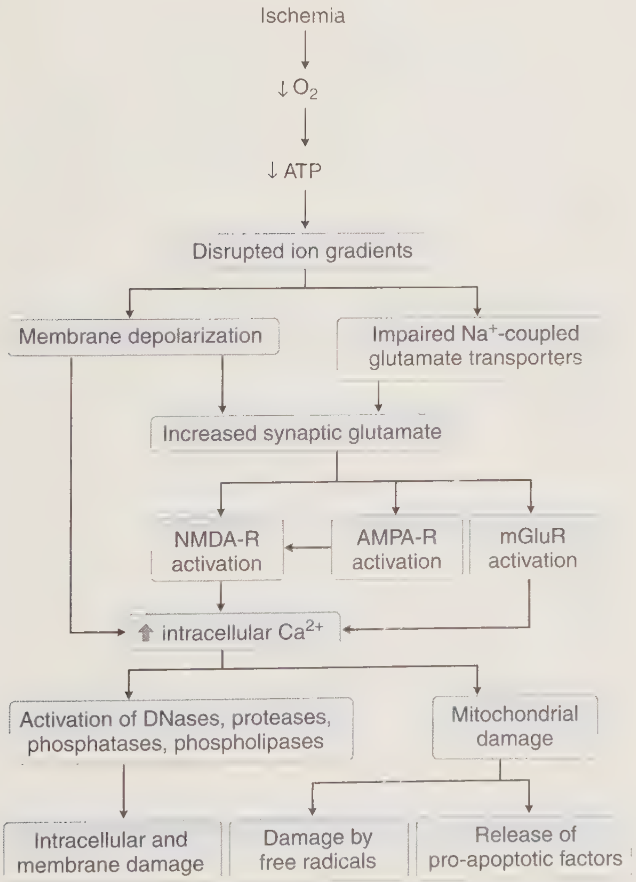

Ischemia
↓ $O_2$
↓ ATP
Disrupted ion gradients
Membrane depolarization
Impaired $Na^+$-coupled glutamate transporters
Increased synaptic glutamate
NMDA-R activation
AMPA-R activation
mGluR activation
↑ intracellular $Ca^{2+}$
Activation of DNases, proteases, phosphatases, phospholipases
Mitochondrial damage
Intracellular and membrane damage
Damage by free radicals
Release of pro-apoptotic factors

**Figure 10-5. Role of Glutamate Receptors in Excitotoxicity.** Although a multiplicity of damaging cellular processes occur as a consequence of the decreased ATP levels that result from impaired oxidative metabolism, only glutamate-mediated processes are depicted here.

AMPA receptors may constitute another pharmacologic target in the treatment of damage secondary to ischemia and excitotoxicity. Although NMDA receptor antagonists protect effectively against only moderate ischemia in animal models, AMPA receptor antagonists prevent delayed neuronal cell death caused by severe ischemia. Severe transient forebrain ischemia alters AMPA receptor subunit expression, resulting in increased $Ca^{2+}$ entry through AMPA receptors. In addition to blocking these $Ca^{2+}$-permeable receptors, AMPA receptor antagonists inhibit membrane depolarization and, thus, reduce activation of NMDA receptors and voltage-activated $Ca^{2+}$ channels. Clinical trials of AMPA receptor antagonists are currently determining the relative contribution of AMPA receptors to ischemic neuronal damage in humans.

Glutamate released during ischemic damage activates metabotropic as well as ionotropic glutamate receptors. Pharmacologic antagonism of the mGluR1 receptor subtype not only facilitates recovery and survival of hippocampal neurons in animal stroke models, but also prevents memory and motor loss caused by trauma. These data suggest that the mGluR1 subunit may represent another target for future pharmacologic intervention (Fig. 10-6).

## HYPERALGESIA

NMDA receptors contribute to hyperalgesia by enhancing synaptic transmission between nociceptive afferent fibers and dorsal horn neurons. As Chapter 15 discusses in more detail, experimental hyperalgesia often involves a phenomenon called "central sensitization." In central sensitization, repeated nociceptive stimuli reduce the $Mg^{2+}$ blockade of NMDA receptors at resting membrane potentials, leading progressively to increasing excitatory postsynaptic potentials. By enhancing the activity of NMDA receptors, and, thereby, the synaptic efficiency of sensory circuits, central sensitization causes hypersensitivity to painful stimuli (Fig. 10-7). Accordingly, NMDA receptor antagonists can both prevent and reverse central sensitization in patients. Many of these antagonists also inhibit a wide range of fast excitatory synaptic pathways in the CNS, however. For this reason, current NMDA receptor drug development focuses on intraspinal or extradural administration of NMDA receptor antagonists to limit the effects of the drug to the dorsal horn of the spinal cord. The high density of kainate receptors in sensory neurons may also modulate transmitter release, providing another future pharmacologic target for the relief of chronic pain.

## EPILEPSY

Seizures can result from overstimulation of glutamatergic pathways, beginning with overactivation of AMPA receptors and progressing to overactivation of NMDA receptors. In animal models, inhibition of AMPA receptor activation prevents seizure onset; whereas, NMDA receptor antagonists decrease seizure intensity and duration. **Lamotrigine,** a drug used to treat refractory complex partial seizures (see Chapter 13, Pharmacology of Abnormal Electrical Activity in the Central

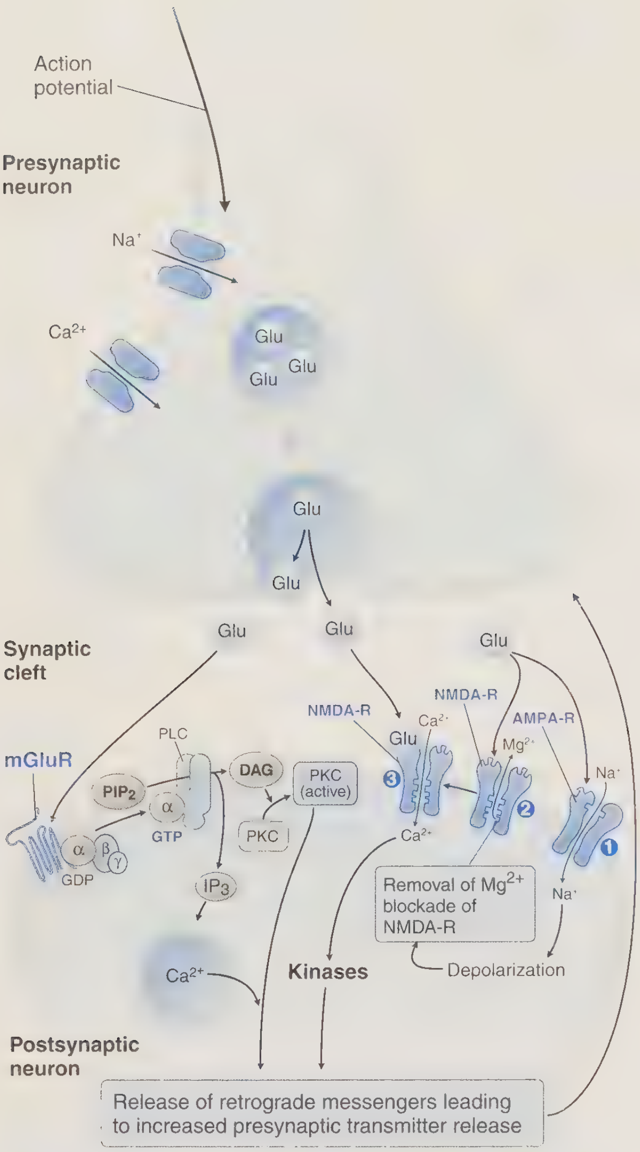

Action potential
Presynaptic neuron
Na+
Ca2+
Glu
Glu
Glu
Glu
Glu
Synaptic cleft
Glu
Glu
Glu
mGluR
PLC
PIP2
α
GTP
α
β
γ
GDP
DAG
PKC (active)
PKC
IP3
Ca2+
NMDA-R
NMDA-R
Ca2+
Glu
AMPA-R
Mg2+
Na+
3
2
1
Ca2+
Removal of Mg2+ blockade of NMDA-R
Na+
Depolarization
Kinases
Postsynaptic neuron
Release of retrograde messengers leading to increased presynaptic transmitter release

**Figure 10-6. Interactions Among Metabotropic, AMPA, and NMDA Classes of Glutamate Receptors.** Action potentials depolarize the plasma membrane of presynaptic neurons, leading to opening of voltage-gated $Ca^{2+}$ channels and, ultimately, to glutamate release into the synaptic cleft. Studies have proposed a "tonic" physiological role for activation of the metabotropic glutamate receptor (mGluR) during low frequency stimulation of postsynaptic neurons by glutamate. In contrast, high-frequency stimulation "phasically" activates AMPA receptors **(1)** and, thereby, induces the prolonged membrane depolarization required to relieve the $Mg^{2+}$ blockade of NMDA receptors **(2).** The activated NMDA receptor **(3)** is then able to activate downstream kinases independently of the mGluR. AMPA-R, AMPA receptor; DAG, diacylglycerol; $IP_3$, inositol-1,4,5-trisphosphate; mGluR, metabotropic glutamate receptor; NMDA-R, NMDA receptor; $PIP_2$, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C.

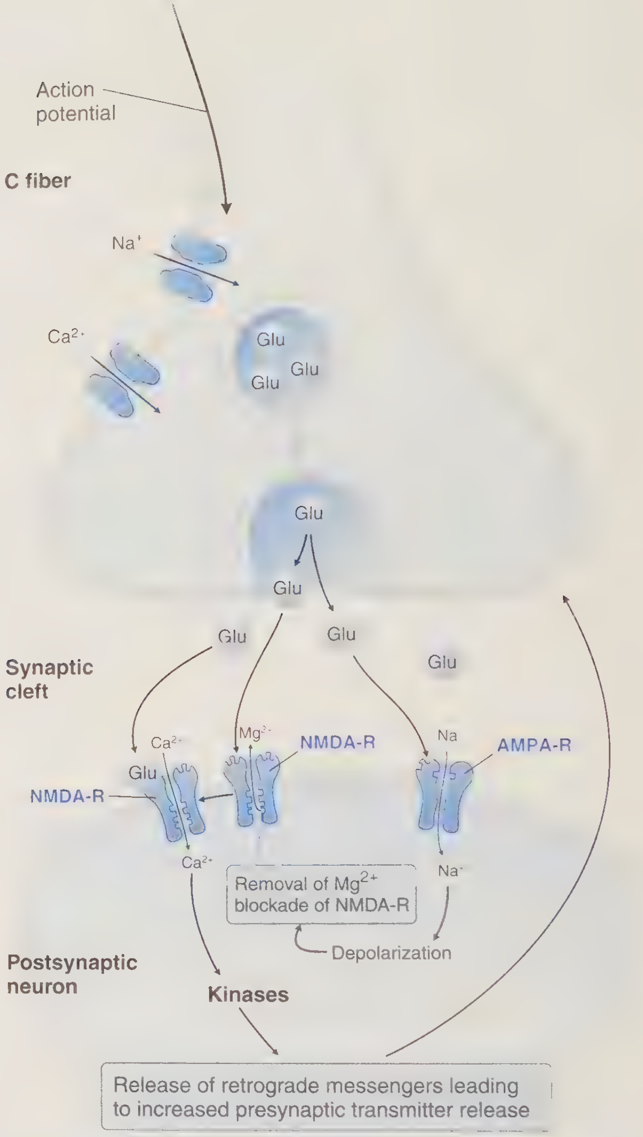

Action potential
C fiber
Na+
Ca2+
Glu
Glu
Glu
Glu
Glu
Synaptic cleft
Glu
Glu
Glu
Ca2+
Mg2+
NMDA-R
Na
AMPA-R
Glu
NMDA-R
Ca2+
Removal of Mg2+ blockade of NMDA-R
Na+
Depolarization
Postsynaptic neuron
Kinases
Release of retrograde messengers leading to increased presynaptic transmitter release

**Figure 10-7. Potential Role of Glutamate Neurotransmission in Hyperalgesia.** Action potentials depolarize the plasma membrane of presynaptic C fibers (unmyelinated axons of pain- and temperature-responsive neurons), leading to opening of voltage-gated $Ca^{2+}$ channels and, ultimately, to glutamate release into the synaptic cleft. Brief pain stimuli release limited quantities of glutamate, which bind to AMPA receptors (AMPA-R) to cause transient depolarizations of neurons in the dorsal horn of the spinal cord. Prolonged pain stimuli allow AMPA receptors to induce membrane depolarizations that are sufficient to relieve the $Mg^{2+}$ blockade of the NMDA receptor (NMDA-R). Repetitive NMDA receptor activation (and, possibly, metabotropic glutamate receptor activation) leads to central "sensitization" and hyperalgesia.

Nervous System), stabilizes the inactivated state of the voltage-gated $Na^+$ channel, and, thereby, reduces membrane excitability, the number of action potentials in a burst, glutamate release, and glutamate receptor activation. The pathogenesis of epilepsy and the associated pharmacologic interventions are discussed further in Chapter 13.

## NEURODEGENERATIVE DISEASES

Excessive glutamate receptor activation may contribute to the pathophysiology of certain neurodegenerative diseases. In ALS, motor neurons degenerate in the ventral horn of the spinal cord, brainstem, and motor cortex, resulting in increasing weakness and atrophy of skeletal muscles. The pathogenesis of this disease and the reasons for the selective pattern of neurodegeneration remain uncertain, but mechanisms currently proposed for cell death in ALS include excitotoxicity and oxidative stress. The CNS areas affected in ALS express diverse populations of AMPA and NMDA receptors as well as glutamate reuptake transporters. Patients with ALS have impaired glutamate transporters in the spinal cord and motor cortex. These abnormal glutamate transporters permit the accumulation of high glutamate concentrations in the synaptic cleft, possibly leading to motor neuron death via excitotoxicity. In addition, plasma and cerebrospinal fluid from individuals with ALS cause cell death when added to neuronal cell cultures; this effect is blocked by both the voltage-gated sodium channel blocker **riluzole** and the AMPA receptor antagonist **CNQX.** Riluzole has cerebroprotective, antiepileptic, and analgesic effects. This drug prolongs survival and decreases disease progression in patients with ALS, at least in part by reducing $Na^+$ conductance, thereby, decreasing glutamate release.

In Parkinson's disease, reduced dopaminergic transmission to the striatum results in the overactivation of glutamatergic synapses in the CNS. Thus, glutamatergic neurotransmission contributes to the clinical signs of Parkinson's disease, as discussed in Chapter 11. The drug **amantadine** is a noncompetitive blocker of NMDA receptor channels, similar in action to memantine. Although amantadine is not an effective treatment as a single agent, the combination of amantadine and **levodopa** reduces the severity of dyskinesia in Parkinson's disease by 60%. It is not clear whether this effect derives solely from NMDA receptor blockade, however.

# PHYSIOLOGY OF THE GABA RECEPTOR

## GABA SYNTHESIS AND DEGRADATION

GABA functions as the primary inhibitory neurotransmitter in the mammalian CNS. The cell membranes of most vertebrate CNS neurons and astrocytes express GABA receptors. GABA receptors are also located outside the CNS on autonomic neurons. The biochemical synthesis of GABA is intertwined with that of glutamate (Fig. 10-2A). Synthesis depends on the activity of the enzyme **glutamic acid decarboxylase** (GAD), which catalyzes the decarboxylation of glutamate to GABA in GABAergic nerve terminals. Thus, the amount of GABA in the brain correlates with the amount of functional GAD. GAD requires pyridoxal phosphate (vitamin $B_6$) as a cofactor.

Termination of GABA action at the synapse depends on the removal of GABA from the extracellular space. Neurons and glia take up GABA via a sodium-GABA cotransporter that generates a passive net inward current. Within cells, GABA metabolism intersects with oxidative carbohydrate metabolism. The widely distributed mitochondrial enzyme GABA-transaminase (GABA-T) catalyzes the conversion of GABA to succinic semialdehyde (SSA), which is subsequently oxidized to succinic acid by SSA dehydrogenase and then enters the Krebs cycle to become α-ketoglutarate. GABA-T then regenerates glutamate from α-ketoglutarate (Fig. 10-2A).

## GABA RECEPTORS

There are two main types of GABA receptors involved in neuronal transmission: the **$GABA_A$** receptor and the **$GABA_B$** receptor. In most regions of the brain, inhibitory postsynaptic potentials (**IPSPs**) consist of a fast and a slow component. *Ionotropic $GABA_A$ receptors generate fast IPSPs, while metabotropic $GABA_B$ receptors mediate slow IPSPs.* This difference in signaling kinetics stems from the mode of coupling between each type of GABA receptor and its ion channel. The $GABA_A$ receptor contains a ligand-gated $Cl^-$ ion channel that opens shortly after GABA binding, while the $GABA_B$ receptor is coupled to ion conductance changes by several biochemical reactions that occur more slowly (see below). As with the nicotinic acetylcholine receptor, the $GABA_A$ receptor is a pentameric glycoprotein composed of a central ion pore surrounded by five receptor subunits, each of which has four membrane-spanning domains. $GABA_A$ receptor activation requires the simultaneous binding of two molecules of GABA, one to each of the receptor's two α subunits (Fig. 10-8).

Although $GABA_A$ receptors contain an intrinsic ion channel, $GABA_B$ receptors are second-messenger–linked, seven transmembrane receptors that activate GTP-binding proteins (G proteins) to open $K^+$ ion channels (Fig. 10-9). $GABA_B$ receptors are expressed at lower levels than $GABA_A$ receptors and exist principally in the spinal cord. Different $GABA_A$ and $GABA_B$ receptor subtypes consist of different subunit combinations with distinct functional and pharmacologic properties. As noted above in the discussion of glutamate receptors, variations in the anatomic distribution of subunits generate a variety of GABA receptor isoforms in different regions of the CNS. The diversity of region-specific channel isoforms allows drugs acting at GABA receptors to affect certain brain regions preferentially, and thus to have specific behavioral effects.

### $GABA_A$ Receptor

The $GABA_A$ receptor contains separate agonist, antagonist, and modulator binding sites that often interact cooperatively (Fig. 10-8). Activation of the $GABA_A$ receptor requires the binding of two GABA molecules, resulting in conformational changes that open the chloride ion channel and allow an inward flow of $Cl^-$ (Fig. 10-10). This outward current, equivalent to a loss of positive charge, *hyperpolarizes* the cell membrane. In addition, the channel opening decreases membrane

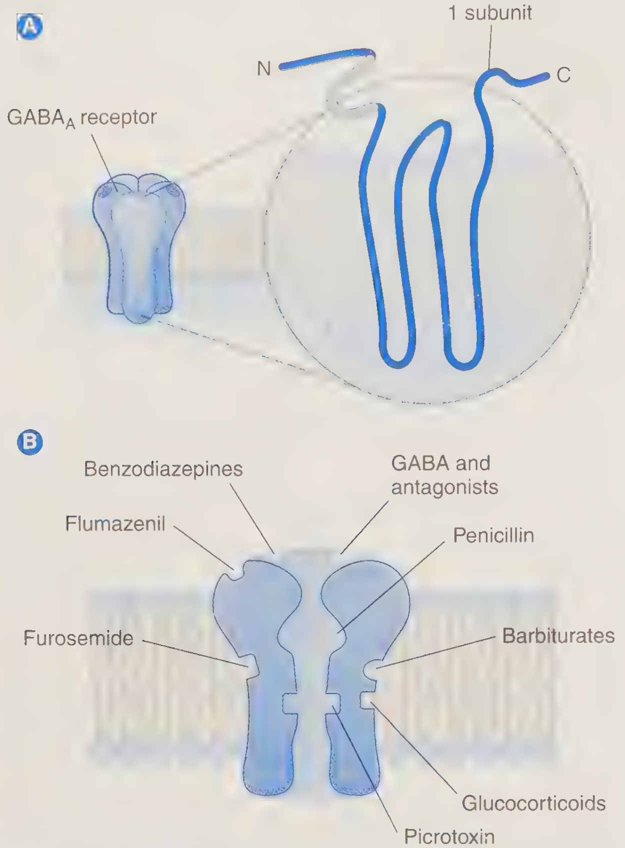


**Figure 10-8. Schematic Representation of the GABA$_A$ Receptor.** **A.** The pentameric structure of the GABA$_A$ receptor. Each receptor is made up of five subunits, and each subunit is of one of three predominant subtypes: $\alpha$, $\beta$, or $\gamma$. Activation requires the binding of two GABA molecules to the receptor, one to each of the two $\alpha$ subunits. Each subunit of the GABA$_A$ receptor has four membrane-spanning regions and a cysteine loop in the extracellular N-terminal domain (depicted as a dashed line). **B.** Major binding sites on the GABA$_A$ receptor. Although there is indirect evidence for the location of many of the drug binding sites that are schematically indicated in this diagram, the definitive localization of these sites remains to be determined.

resistance and, thereby, increases the inward current required to depolarize the membrane (see below).

There are two main patterns of GABAergic transmission: burst and tonic firing. During burst (or "phasic") firing, the stimulated presynaptic membrane releases "quanta" of transmitter molecules through exocytosis of synaptic vesicles, resulting in large-amplitude IPSPs. In contrast, tonic (or "basal") firing results in low levels of released transmitter that cause a baseline inhibitory current at postsynaptic membranes. The mechanism of this tonic release of GABA remains unknown. The inhibitory effects of burst and tonic firing depend in part on the cell membrane potential. Because the $Cl^-$ equilibrium potential ($E_{Cl} \sim -70$ mV) is close to the resting membrane potential of most neurons ($V_m \sim -65$ mV), there is little net $Cl^-$ flow through open $Cl^-$ channels in a cell at rest. These open channels still have an inhibitory effect on neuronal excitability; however, because they function as "leaks" that decrease the membrane resistance. This decrease in membrane resistance allows open $Cl^-$ channels to decrease the change in membrane potential caused by an excitatory synaptic current, an effect called **shunting.** By this mechanism, activation of the GABA$_A$ receptor results in a stronger inhibitory response, characterized by a larger chloride influx and a diminished excitatory depolarization, whenever an excitatory input depolarizes the membrane.

## GABA$_B$ Receptor

In contrast to the fast IPSPs mediated by GABA$_A$ receptors, GABA$_B$ receptors produce slow, prolonged inhibitory signals. GABA$_B$ receptors also regulate presynaptic neurotransmitter release. GABA binding to the GABA$_B$ receptor activates G proteins that open a $K^+$ channel, resulting in $K^+$ efflux and membrane hyperpolarization. In addition, presynaptic GABA$_B$ receptors act as autoreceptors to decrease neurotransmitter release, although the exact mechanisms by which this occurs remain undetermined. One possible mechanism is that activation of $K^+$ channels results in an outward current that shortens the duration of the presynaptic action potential and thereby reduces $Ca^{2+}$ entry; another is that GABA$_B$ receptor activation results in G protein-mediated modification of the $Ca^{2+}$ channel per se and, thereby, reduces $Ca^{2+}$ influx (Fig. 10-9). By either

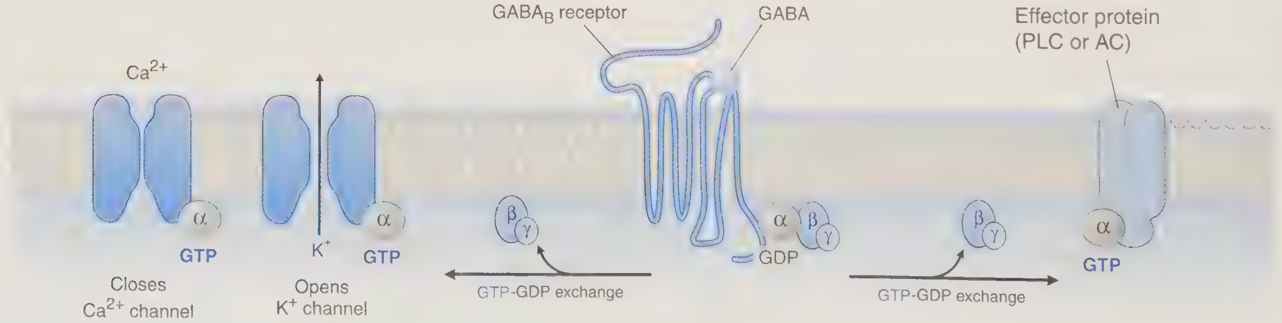


**Figure 10-9. Downstream Signaling of the GABA$_B$ Receptor.** GABA$_B$ receptors activate G proteins that are either coupled to $K^+$ or $Ca^{2+}$ channels directly (leftward arrow) or linked to second messenger systems such as adenylyl cyclase (AC) or phospholipase C (PLC) (rightward arrow). The increased $K^+$ efflux leads to slow, long-lasting inhibitory postsynaptic potentials. The reduced $Ca^{2+}$ influx may enable GABA$_B$ autoreceptors to inhibit presynaptic neurotransmitter release.

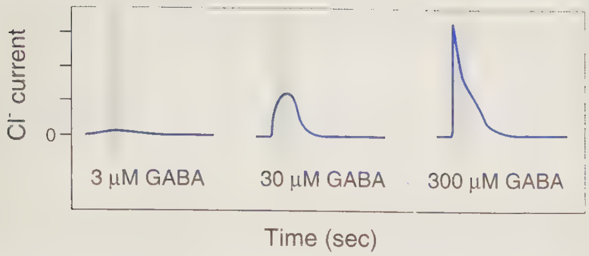


**Figure 10-10. Effects of GABA on $GABA_A$-Mediated Chloride Conductance.** Increasing concentrations of GABA induce both greater $Cl^-$ currents and more rapid receptor desensitization. The latter phenomenon can be observed as the rapid decline from the peak current during continuous exposure to 300 µM GABA (right panel). In each panel, the shaded bar indicates the time during which GABA was applied.

mechanism, decreased presynaptic calcium entry reduces neurotransmitter release. Unlike $GABA_A$ receptors, $GABA_B$ receptors are not stimulated by tonic firing.

### $GABA_C$ Receptor

A third type of GABA receptor, the $GABA_C$ receptor, structurally resembles the ionotropic $GABA_A$ receptor. Both receptors consist of five subunits that form a transmitter-gated $Cl^-$ ion channel. The $GABA_C$ receptor is found at synapses between spinal cord interneurons, in the brain, and in the retina.

The $GABA_C$ receptor has pharmacologic properties that distinguish it from other GABA receptors. Neither $GABA_A$ receptor modulators, such as barbiturates and benzodiazepines, nor $GABA_B$ receptor agonists, such as **baclofen,** bind to $GABA_C$ receptors. Instead, the $GABA_C$ receptor is activated by certain GABA analogues, and GABA itself is ten times more potent at $GABA_C$ than at $GABA_A$ receptors. Because the current knowledge regarding $GABA_C$ receptor modulators is limited, this receptor subtype is not known to be a target for any clinically approved drugs.

## PHARMACOLOGIC CLASSES AND AGENTS AFFECTING GABAERGIC NEUROTRANSMISSION

All current therapeutics that act on GABAergic neurotransmission activate inhibitory GABAergic pathways and thereby depress the CNS. Modulation of GABAergic transmission can occur either presynaptically or postsynaptically. Drugs acting at presynaptic sites primarily target GABA synthesis, degradation, and reuptake rather than the GABA receptors per se. For example, **gabaculine** inhibits GABA-T, the enzyme responsible for the conversion of GABA to succinic semialdehyde, resulting in increased levels of GABA and anticonvulsant effects. Drugs acting postsynaptically affect the GABA receptor directly, either by binding to the GABA binding site or by allosterically altering GABA binding (Table 10-3). Each of the

**TABLE 10-3** Partial List of Agents That Modulate GABAergic Transmission

| DRUG CLASS | PRESUMED MECHANISM | EFFECTS |
|---|---|---|
| **GABA Synthesis** | | |
| Allylglycine | Inhibits glutamic acid decarboxylase | Convulsant |
| Isoniazid | Inhibits pyridoxal kinase (antivitamin $B_6$ effect) | Convulsant at high doses |
| **GABA Release** | | |
| Tetanus toxin | Inhibits GABA and glycine release | Convulsant |
| **GABA Metabolism** | | |
| Gabaculine | Inhibits GABA transaminase | Anticonvulsant |
| **GABA Receptor Agonists** | | |
| Muscimol | $GABA_A$ receptor agonist | Mimics psychosis |
| Baclofen | $GABA_B$ receptor agonist | Muscle relaxant |
| **$GABA_A$ Receptor Antagonists** | | |
| Bicuculline | Competitive antagonist | Convulsant |
| Picrotoxin | Noncompetitive antagonist, pore blocker, occludes the chloride channel | Convulsant |
| **$GABA_A$ Receptor Modulators** | | |
| Benzodiazepines | Potentiate GABA binding | Anticonvulsant, anxiolytic |
| Barbiturates | Increase GABA efficacy, weak agonist | Anticonvulsant, anesthetic |

three main GABA receptor types has a distinct pharmacology, but because most drugs in use today affect the $GABA_A$ receptor, the following discussion of GABA pharmacology focuses on modulators of this receptor.

Sedative/anxiolytic drugs reduce an individual's level of anxiety and responsiveness to stimulation; whereas, hypnotic drugs induce sleep. Hypnotics cause deeper CNS depression than sedatives, although at high doses most sedative drugs have a hypnotic effect. Both sedative/anxiolytic and hypnotic drugs exert their effects through activation of $GABA_A$ receptors, which have, in addition to the GABA binding site, a barbiturate binding site (where volatile and most intravenous general anesthetics and CNS depressants may also bind), a benzodiazepine binding site, and a steroid binding site (where certain intravenous general anesthetics such as **alphaxalone** bind) (Fig. 10-8). The rest of this chapter discusses how the barbiturates and benzodiazepines achieve their pharmacologic effects through the modulation of $GABA_A$ receptor physiology.

## $GABA_A$ RECEPTOR AGONISTS AND ANTAGONISTS

Activation of the $GABA_A$ receptor by GABA results in a concentration-dependent $Cl^-$ influx and membrane hyperpolarization, although higher GABA concentrations also cause a faster rate of decline in the $Cl^-$ current because of receptor desensitization (Fig. 10-10). Certain $GABA_A$ receptor agonists, such as **muscimol,** activate the receptor by binding directly to the GABA binding site. $GABA_A$ receptor agonists can also indirectly amplify the effects of endogenous GABA by increasing the affinity of the receptor for GABA or by enhancing the effect of GABA receptor activation on chloride flux. For example, such agents could increase the length of time during which the chloride channel is open after GABA binding.

Because of their widespread activation of $GABA_A$ receptors, $GABA_A$ receptor full agonists cause marked side effects, such as sedation and ataxia, as well as tolerance and physical dependence. Recent drug development efforts have been directed toward the development of $GABA_A$ receptor partial agonists, which may cause fewer side effects. Although partial agonists have shown anxiolytic and anticonvulsant effects, they do not induce other effects such as sedation, muscle relaxation, and physical dependence. Presumably, the latter effects require full receptor activation.

$GABA_A$ receptor antagonists inhibit GABAergic function through a variety of mechanisms, including direct competition for the GABA binding site, indirect modification of the GABA binding site by binding to another region of the receptor, or occlusion of the ion channel. **Bicuculline** inhibits GABA-induced membrane hyperpolarization by competing for the GABA binding site. Higher concentrations of GABA administered concomitantly with bicuculline can overcome the antagonist effect, demonstrating the competitive nature of bicuculline's antagonism. In contrast, the noncompetitive antagonist **picrotoxin** binds within the chloride ion channel rather than at the GABA binding site; by occluding the $Cl^-$ channel, this plant toxin (related to compounds used in certain insecticides) has a convulsant effect.

Although $GABA_A$ receptor agonists and antagonists with a wide range of mechanisms are used in research, the major mechanism employed by most drugs in clinical practice is not to activate the GABA receptor directly, to alter GABA metabolism, or to inhibit GABA reuptake. Instead, the two major classes of sedative/anxiolytic drugs that manipulate GABAergic transmission, the benzodiazepines and the barbiturates, exert their effects by altering the interaction between the $GABA_A$ receptor and its endogenous ligand.

## BARBITURATES

**Barbiturates** can induce degrees of CNS depression ranging from mild sedation to general anesthesia. These drugs increase inhibitory transmission throughout the CNS by acting presynaptically as well as postsynaptically, primarily targeting $GABA_A$ receptors. The presynaptic sites of action are largely located in the spinal cord; the postsynaptic sites include multiple sites in the brain (cortex, thalamus, cerebellum) and brainstem (cuneate nucleus, substantia nigra, reticular activating system). The site of drug action determines the physiologic response. For example, increased GABAergic transmission in the brainstem suppresses the reticular activating system (discussed in Chapter 6, Principles of Nervous System Physiology and Pharmacology), causing sedation, amnesia, and loss of consciousness. Heightened GABAergic transmission at motor neurons in the spinal cord relaxes muscles and suppresses reflexes.

### Mechanism of Action

Barbiturates reduce neuronal excitability through an array of mechanisms that can be subdivided into two main groups: increased neuronal inhibition and decreased neuronal excitation. Increased inhibition occurs primarily through modulation of and, to a much lesser extent, direct activation of the $GABA_A$ receptor. The differences among the barbiturates in their effects on $GABA_A$ receptors give rise to barbiturate subclasses with distinct clinical effects and uses. For example, anesthetic barbiturates, such as **pentobarbital,** directly activate $GABA_A$ receptors to a greater extent than do anticonvulsant barbiturates, such as **phenobarbital,** and hence, exert anticonvulsant effects only at deeply sedating doses. In contrast, the anticonvulsant effects of anticonvulsant barbiturates occur at doses that produce only minimal sedation. Studies have suggested that the extent of direct $GABA_A$ receptor activation depends, at least in part, on the drug's interactions with specific amino acids in the receptor subunits. The therapeutic index of phenobarbital is greater than that of pentobarbital, because the latter's anticonvulsant effect is accompanied by suppression of spontaneous neuronal activity (indicated by its sedative effects). Thus, pentobarbital is more likely to induce profound, and potentially fatal, CNS depression (Table 10-4).

The direct agonist activity of either an anesthetic or an anticonvulsant barbiturate alone does not cause nearly the degree of membrane hyperpolarization that results from simultaneous administration of barbiturate and GABA. Rather, *the major action of the barbiturates is to enhance the efficacy of GABA by greatly increasing the duration of* $Cl^-$ *channel opening, thus permitting a much greater influx of* $Cl^-$ *ions for each activated channel* (Fig. 10-11). This leads to a greater degree of hyperpolarization and to decreased excitability of the

**TABLE 10-4** Comparison of Pentobarbital and Phenobarbital

| | PENTOBARBITAL | PHENOBARBITAL |
|---|---|---|
| Routes of administration | Oral, IM, IV, rectal | Oral, IM, IV |
| Duration of action | Short-acting (1–4 h) | Long-acting (days) |
| Suppression of spontaneous neuronal activity | Yes | Minimal |
| Activity at $GABA_A$ receptor | Major: Increases efficacy of GABA by increasing open time of $Cl^-$ channel.<br>Minor: Direct $GABA_A$ receptor activation | Increases efficacy of GABA by increasing open time of Cl channel |
| Activity at glutamate receptor | Noncompetitive antagonist at AMPA receptor (2–3 times more potent than phenobarbital) | Noncompetitive antagonist at AMPA receptor |
| Therapeutic uses | Preoperative sedation<br>Emergency treatment for seizures | Anticonvulsant |

target cell. Therefore, the main effects elicited by barbiturates result from their synergistic relationship with GABA: concomitant barbiturate and GABA administration hyperpolarizes the membrane to a greater extent than the sum of the hyperpolarization responses caused by each receptor agonist separately.

Barbiturates affect not only $GABA_A$ receptors, but also receptors involved in excitatory neurotransmission. At subanesthetic concentrations, barbiturates decrease activation of the AMPA receptor by glutamate (see Fig. 10-3), thereby reducing both membrane depolarization and neuronal excitability. At anesthetic concentrations, such barbiturates as pentobarbital also decrease the activity of voltage-dependent $Na^+$ channels, consequently inhibiting high-frequency neuronal firing. Inhibition of $Na^+$ channels in the brainstem's arousal pathway, the reticular activating system, reduces neuronal activity and further suppresses wakefulness.

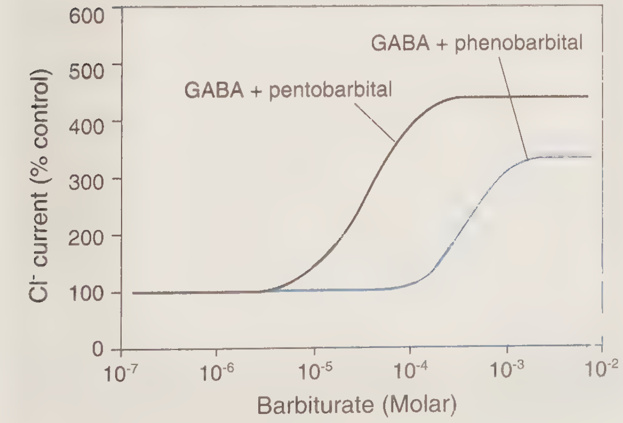


**Figure 10-11. Effect of Barbiturates on GABA-Induced $GABA_A$-Mediated Chloride Currents.** Addition of either pentobarbital or phenobarbital increases the current evoked by 10 μM GABA to a much greater level than that elicited by 10 μM GABA alone. Compared to phenobarbital, pentobarbital has a higher efficacy (indicated by the greater height of its concentration-response curve) and a greater potency (indicated by the leftward position of its curve). However, the $EC_{50}$ of GABA alone ($EC_{50} \approx 20$ μM) is less than that of GABA + pentobarbital ($EC_{50} \approx 40$ μM) and that of GABA + phenobarbital ($EC_{50} \approx 400$ μM). Therefore, these barbiturates increase the efficacy but not the potency of GABA. 100% control refers to the current evoked by 10 μM GABA alone.

## Clinical Applications

Before the advent of the benzodiazepines, the sedative/hypnotic effects of barbiturates were commonly used to treat insomnia or anxiety. Benzodiazepines have now replaced barbiturates in most clinical applications, because benzodiazepines cause less tolerance, have fewer withdrawal symptoms, and induce less profound effects on drug-metabolizing enzymes. Most significantly, barbiturates have a lower selectivity and a greater toxic potential than benzodiazepines. As discussed above, high concentrations of barbiturates not only synergize with GABA at $GABA_A$ receptors, but also independently activate $GABA_A$ receptor $Cl^-$ channels and inhibit excitatory neurotransmission. Nonetheless, certain barbiturates are still used occasionally as anesthetic and antiepileptic agents (Table 10-5).

The lipid-soluble barbiturates, such as **thiopental** and **methohexital,** induce general anesthesia. These drugs rapidly enter the brain after intravenous administration and are then redistributed among the less vascularized tissues (see Chapter 14, General Anesthetic Pharmacology). This pattern of distribution dictates the short duration of action that renders these drugs useful induction agents for general anesthesia. **Propofol** is another $GABA_A$ receptor activator used for anesthesia induction and maintenance, now more frequently than barbiturates. It has a faster onset of action (approximately 30 seconds) and a shorter duration of action (3–10 minutes) than thiopental. These anesthetic drugs are discussed further in Chapter 14.

**TABLE 10-5** Clinical Uses and Relative Duration of Action of Several Barbiturates

| BARBITURATE | CLINICAL USES | DURATION OF ACTION |
|---|---|---|
| Thiopental | Anesthesia induction and short-term maintenance, emergency seizure treatment | Ultrashort-acting (5–15 minutes) |
| Methohexital | Anesthesia induction and short-term maintenance | |
| Pentobarbital | Insomnia, preoperative sedation, emergency seizure treatment | Short-acting (3–8 hours) |
| Secobarbital | Insomnia, preoperative sedation, emergency seizure treatment | |
| Amobarbital | Insomnia, preoperative sedation, emergency seizure treatment | |
| Phenobarbital | Treatment of seizures, status epilepticus | Long-acting (days) |

A barbiturate's duration of action is determined by the rapidity with which it is redistributed from the brain to other, less vascular compartments, particularly to muscle and fat.

Barbiturates also serve as effective antiepileptics. As discussed in Chapter 13, seizures are characterized by rapidly depolarizing CNS neurons that repeatedly fire action potentials. Such barbiturates as **phenobarbital** hyperpolarize the neuron by amplifying GABA-mediated synaptic inhibition; thereby, decreasing neuronal excitability and raising the seizure threshold. These drugs further decrease neuronal excitability by inhibiting AMPA receptor-mediated excitatory transmission. Phenobarbital is used to treat partial and tonic–clonic seizures (see Chapter 14).

## Pharmacokinetics and Metabolism

A barbiturate's ability to cross the blood–brain barrier and enter the CNS is largely determined by its lipid solubility. Consequently, termination of the drug's acute CNS effects depends primarily on its redistribution from the brain, first to such highly perfused areas as the splanchnic circulation, then to skeletal muscle, and, finally, to poorly perfused adipose tissue. As mentioned above, bolus administration of a barbiturate that redistributes rapidly causes only a short-lived effect on the CNS. Chronic administration of the lipophilic barbiturates may have a prolonged effect because of the high capacity of adipose tissue for these drugs, which leads to a high volume of distribution and, consequently, to a long elimination half-life.

Barbiturates that are lipophilic undergo extensive hepatic metabolism before renal excretion (Table 10-6). Elderly patients (who often have impaired liver function) and patients with severe liver disease have reduced barbiturate clearance, and even normal doses of sedative–hypnotics may have significantly greater CNS effects in these patients. In the case presented at the start of this chapter, S.B.'s advanced age contributed to the CNS depression he experienced when he took phenobarbital, one of the few barbiturates that undergoes both renal and hepatic clearance. Approximately 25% of a phenobarbital dose is cleared as the unchanged drug in the urine, while the liver metabolizes the remaining 75%. Because such acidic compounds as phenobarbital are excreted faster in alkaline than in acidic urine, the medical team treating S.B. gave him intravenous sodium bicarbonate to increase phenobarbital clearance.

Chronic barbiturate use up-regulates the expression of hepatic cytochrome P450 oxidases, thereby accelerating the metabolism of all substrates for these enzymes. Thus, barbiturates enhance the metabolism of other sedative/hypnotics, of drugs such as phenytoin, digoxin, and oral contraceptives, of endogenous substances such as steroid hormones, bile salts, and cholesterol, and of vitamins D and K.

**TABLE 10-6** Metabolism of Barbiturates

| BARBITURATE | METABOLISM |
|---|---|
| Most barbiturates (lipophilic) | Nearly complete hepatic metabolism and/or conjugation |
| Phenobarbital | 75% hepatic metabolism and/or conjugation 25% excreted unchanged in urine |
| Aprobarbital | Excreted unchanged in urine<br>Alkalinization of urine increases excretion because aprobarbital is a weak acid |

## Toxicity

The multiplicity of sites at which barbiturates act, coupled with their ability to activate independently the $GABA_A$ receptor $Cl^-$ channel at high doses, contributes to the relatively low selectivity and therapeutic index of these drugs. Unlike benzodiazepines, high doses of barbiturates can cause a potentially fatal level of CNS depression. In addition, the concomitant administration of barbiturates and other CNS depressants, such as ethanol, results in CNS depression more severe than that caused by barbiturates alone. As seen in the case of S.B., a patient suffering from barbiturate poisoning experiences severe respiratory depression; this effect may be accompanied by cardiovascular depression, leading to a condition resembling shock. Had S.B. not received timely and adequate treatment, respiratory depression, loss of brainstem vasomotor control, and direct myocardial depression could have resulted in deep coma and death. Treatment of these cases involves the maintenance of adequate respiratory and cardiovascular support, the administration of activated charcoal to absorb recently ingested drug, and alkalinization of the urine to increase barbiturate clearance (see Chapter 47, Principles of Toxicology).

## Tolerance and Dependence

Repeated and extended misuse of sedative–hypnotic drugs induces tolerance and physiologic dependence. Prolonged barbiturate use greatly increases the activity of the hepatic cytochrome P450 system and speeds barbiturate breakdown, thereby contributing to the development of tolerance to barbiturates, other sedative/hypnotics, and ethanol. Chronic use of sedative–hypnotics can lead to physiologic dependence, requiring the continued presence of drug to prevent a withdrawal syndrome characterized by tremors, anxiety, insomnia, and CNS excitability. If left untreated, these withdrawal signs may progress to seizures and cardiac arrest (see Chapter 16).

# BENZODIAZEPINES

As with barbiturates, benzodiazepines modulate $GABA_A$ receptor activity and have sedative, hypnotic, muscle relaxant, and anxiolytic effects. At high doses, benzodiazepines can cause hypnosis and stupor. However, the preferential use of benzodiazepines over barbiturates as antiepileptics, hypnotics, anxiolytics, and muscle relaxants stems in part from the fact that these drugs—when used alone—rarely cause fatal CNS depression.

## Mechanism of Action

As noted above, barbiturates increase the *duration* of $Cl^-$ channel opening. In contrast, benzodiazepines increase the *frequency* of channel opening without extending the length of the channel open period. Like barbiturates, benzodiazepines do ultimately cause increased $Cl^-$ influx and membrane hyperpolarization, and, therefore, decrease the excitability of the target cell. This increase in channel opening frequency is caused by benzodiazepine-induced enhancement of the binding affinity between GABA and the $GABA_A$ receptor. In other words, benzodiazepines shift the GABA concentration-effect curve to the left (Fig. 10-12). We can summarize the major actions of these two important drug classes by stating that, *at the $GABA_A$ receptor, barbiturates increase the efficacy of GABA; whereas, benzodiazepines increase the potency of GABA.*

Benzodiazepines cannot elicit a response greater than that caused by maximal GABA concentrations because, unlike barbiturates, benzodiazepines have no intrinsic agonist activity; that is, benzodiazepines alone cannot open the chloride channel. This property greatly reduces the potential for fatalities associated with benzodiazepine overdose. However, the margin of safety of benzodiazepines does decrease when the drug is administered concomitantly with other sedative/hypnotics, in part because of the reciprocal coupling among $GABA_A$ binding sites for barbiturates, benzodiazepines, and $GABA_A$ agonists. The CNS depression associated with benzodiazepine overdose can be alleviated by a benzodiazepine antagonist such as **flumazenil.** Although flumazenil has no clinical effect on its own, the drug antagonizes the effects of benzodiazepine administration by competitively blocking benzodiazepine binding to the $GABA_A$ receptor (Fig. 10-8). In patients with benzodiazepine dependence, flumazenil can cause a severe withdrawal syndrome. This drug does not block the effects of barbiturates or ethanol.

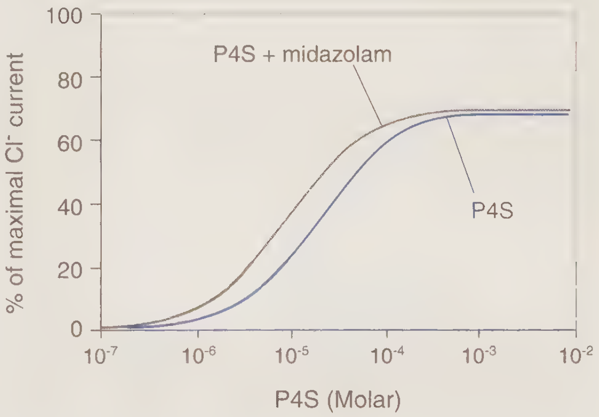


**Figure 10-12. The Benzodiazepine Midazolam Increases the Potency But Not the Efficacy of an Agonist at $GABA_A$ Receptors.** P4S is a $GABA_A$ receptor partial agonist. The combination of midazolam and P4S causes a leftward shift in the concentration-effect curve, indicating that the benzodiazepine increases the potency of the $GABA_A$ partial agonist. Midazolam does not alter the efficacy of P4S, however, because the maximal heights of the two curves are not significantly different. One hundred percent of maximal $Cl^-$ current refers to the current evoked by a full agonist. (To determine whether a drug increases efficacy as a mechanism of receptor modulation, a partial agonist, rather than a full agonist, must be used. By definition, full agonists achieve maximum efficacy as single agents; therefore, the addition of a positive modulator to a full agonist will enhance only effects that are produced at submaximal agonist concentrations. The result appears as a leftward shift of the concentration-response curve, classically interpreted as an increase in potency, but in reality, this effect is merely an artifact from the incorrect use of a full agonist.)

## Clinical Applications

Benzodiazepines are used as anxiolytics, hypnotics, antiepileptics, muscle relaxants, and prophylactic drugs against the symptoms of ethanol withdrawal (Table 10-7). Benzodiazepines achieve an anxiolytic effect by inhibiting synapses in the limbic system, a CNS region associated with the control of emotional behavior and characterized by a high density of $GABA_A$ receptors. Such benzodiazepines as **diazepam** and **alprazolam** are used to mitigate chronic, severe anxiety, and the anxiety associated with some forms of depression and schizophrenia. Because of the potential for the development of tolerance, dependence and addiction, benzodiazepines should be used only intermittently.

Although all the benzodiazepines have a sedative effect, they differ in their rate of onset, duration of effect, and tendency to cause rebound insomnia when used as hypnotics. The sedative effect of benzodiazepines includes decreased ideation and responsiveness to constant stimulation. At higher doses, benzodiazepines induce sleep by facilitating sleep onset and increasing the overall duration of sleep. These drugs also alter the proportion of the various sleep stages: they increase the length of stage 2 non-REM sleep (the light sleep that normally comprises approximately half of sleeping time), and decrease the lengths of REM sleep (the period characterized by frequent dreams) and slow-wave sleep (the deepest level of sleep). These changes may be blunted by the development of tolerance after extended exposure to benzodiazepines. Although an increase in the duration of REM sleep occurs after discontinuation of hypnotic drugs, the drug-induced changes in sleep pattern do not adversely affect pituitary or adrenal hormone secretion. In a healthy individual, hypnotic doses of benzodiazepines induce respiratory changes comparable to those present during natural sleep, and do not cause significant cardiovascular changes. Patients with either pulmonary or cardiovascular disease may experience significant respiratory or cardiovascular depression, however, because of medullary depression from even therapeutic doses of these drugs.

**Flurazepam** is a long-acting benzodiazepine that facilitates sleep onset and maintenance and increases sleep duration. Although it does not cause significant rebound insomnia, its long elimination half-life (about 74 hours) and the accumulation of active metabolites may cause daytime sedation. **Triazolam** is a fast-onset benzodiazepine that also decreases the time needed to fall asleep. Intermittent rather than chronic administration of this drug is recommended to lessen the rebound insomnia associated with its discontinuation.

The antiepileptic effects of benzodiazepines stem from their ability to augment GABA-mediated membrane hyperpolarization and, thus, to decrease the neuronal excitability that contributes to seizure onset. Certain drugs in this class, such as diazepam and **clonazepam,** have selective effects on the CNS, so that their anticonvulsant effects are not accompanied by significant psychomotor impairment. These drugs are discussed at greater length in Chapter 13.

Benzodiazepines reduce skeletal muscle spasticity by increasing the activation of inhibitory GABAergic interneurons in the spinal cord. High doses of these drugs can also inhibit neuromuscular transmission. Diazepam is used to alleviate muscle spasms caused by physical trauma as well as the muscle spasticity associated with neuromuscular degenerative disorders such as multiple sclerosis. The doses required for these effects also result in sedation, however.

Although benzodiazepines, such as diazepam, can supplement the anesthetic effects of other drugs, such as thiopental or inhaled anesthetics, they cannot induce full surgical anesthesia as single agents. However, such a combinatorial

TABLE 10-7 Clinical Uses and Relative Duration of Action of Several Benzodiazepines

| BENZODIAZEPINE | CLINICAL USES | DURATION OF ACTION |
|---|---|---|
| Midazolam | Preanesthetic, IV general anesthetic | Short-acting (3–8 hours) |
| Clorazepate | Anxiety disorders, seizures | Short-acting (3–8 hours) |
| Alprazolam | Anxiety disorders, phobias | Intermediate-acting (10–20 hours) |
| Lorazepam | Anxiety disorders, status epilepticus, IV general anesthetic | Intermediate-acting (10–20 hours) |
| Chlordiazepoxide | Anxiety disorders, alcohol withdrawal | Long-acting (1–3 days) |
| Clonazepam | Seizures | Long-acting (1–3 days) |
| Diazepam | Anxiety disorders, status epilepticus, muscle relaxant, IV general anesthetic, alcohol withdrawal | Long-acting (1–3 days) |
| Triazolam | Insomnia | Short-acting (3–8 hours) |
| Estazolam | Insomnia | Intermediate-acting (10–20 hours) |
| Temazepam | Insomnia | Intermediate-acting (10–20 hours) |
| Flurazepam | Insomnia | Long-acting (1–3 days) |
| Quazepam | Insomnia | Long-acting (1–3 days) |

approach to anesthesia permits the use of lower individual drug doses and enhances control of side effects. Benzodiazepines are adequate as sedatives for brief, uncomfortable procedures associated with little sharp pain, such as endoscopy, and are often given to reduce preoperative anxiety.

### Pharmacokinetics and Metabolism

The lipophilic nature of benzodiazepines explains their rapid and complete absorption after oral administration. Although these drugs and their active metabolites are bound to plasma proteins, there have been no clinically significant reports of competition with other protein-bound drugs. Benzodiazepines are metabolized by hepatic microsomal enzymes and subsequently excreted in the urine as glucuronides or oxidized metabolites. Unlike chronic barbiturate exposure, prolonged benzodiazepine administration does not induce hepatic drug-metabolizing enzyme activity. Because of the important role of hepatic enzymes in metabolizing these drugs, the elderly, the very young, and patients with severe liver disease may experience prolonged effects from benzodiazepine administration. Furthermore, because benzodiazepine metabolism can generate active metabolites with longer half-lives than that of the original drug, the pharmacodynamic effects may extend beyond the expected duration of action.

### Toxicity

The safety of benzodiazepines derives from the inability of these drugs to activate the $GABA_A$ receptor in the absence of endogenous GABA. High doses of benzodiazepines rarely cause death unless administered with other drugs, such as ethanol, CNS depressants, opioid analgesics, or tricyclic antidepressants. One possible cause of the enhanced CNS depression seen with concomitant ethanol and benzodiazepine use is the ethanol-mediated inhibition of hepatic microsomal enzymes. This effect, which occurs when ethanol is administered over a short period of time, is thought to decrease benzodiazepine clearance and, thus, lead to increased CNS depression.

### Tolerance and Dependence

Chronic benzodiazepine use induces tolerance, which is manifested as a decrease in the efficacy of both barbiturates and benzodiazepines. Animal models suggest that benzodiazepine tolerance results from decreased expression of benzodiazepine ($GABA_A$) receptors. Another proposed mechanism for tolerance involves uncoupling of the benzodiazepine binding site from the GABA site. Sudden cessation after chronic benzodiazepine administration can result in a withdrawal syndrome characterized by confusion, anxiety, agitation, and insomnia.

## NONPRESCRIPTION USES OF DRUGS THAT ALTER GABA PHYSIOLOGY

### Ethanol

Ethanol acts as an anxiolytic and sedative by causing CNS depression, but not without significant potential toxicity. Although the molecular targets of ethanol remain uncertain, it is thought that the drug achieves some of its effects through activation of $GABA_A$ receptors. As with barbiturates, ethanol seems to increase $GABA_A$-mediated $Cl^-$ influx and to inhibit the excitatory effects of glutamate. Although barbiturates inhibit AMPA receptor activation, ethanol inhibits NMDA receptor activation. As noted above, ethanol interacts synergistically with such other drugs as sedatives, hypnotics, antidepressants, anxiolytics, anticonvulsants, and opioids. In the introductory case, the ethanol ingested by S.B. accelerated the progression of his CNS depression.

Ethanol tolerance and dependence are associated with a range of changes in $GABA_A$ receptor function. In animal models, chronic ethanol administration blunts the ethanol-mediated potentiation of GABA-induced $Cl^-$ influx in the cerebral cortex and cerebellum. This tolerance to ethanol occurs without a change in the number of $GABA_A$ receptors, but chronic ethanol exposure does alter $GABA_A$ receptor subunit expression in these areas of the brain. Modifications in the subunit composition of $GABA_A$ receptors may, therefore, be responsible for the changes in receptor function associated with chronic ethanol use. Other mechanisms proposed for the development of tolerance to ethanol include post-translational modifications of $GABA_A$ receptors or changes in second messenger systems; for example, alterations in the expression patterns of different isoforms of protein kinase C (PKC). The upregulation of NMDA receptor expression that occurs with prolonged ethanol use may account for the hyperexcitability associated with ethanol withdrawal. Benzodiazepines, such as **diazepam** and **chlordiazepoxide,** reduce the tremors, agitation, and other effects of acute alcohol withdrawal. Although these effects and mechanisms could theoretically explain the development of ethanol tolerance, it should be noted that they are all derived from cellular or animal models of chronic ethanol use and may not translate directly to human pharmacology.

### Chloral Hydrate and Flunitrazepam

**Chloral hydrate** is an older sedative–hypnotic rarely used today to alleviate insomnia. It has occasionally been employed to incapacitate individuals against their will; for example, to facilitate the commission of a crime. **Flunitrazepam** (Rohypnol) is a fast-acting benzodiazepine that can cause amnesia and, thereby, prevent an individual's recall of events that occurred under the drug's influence. This drug has been reported to facilitate "date rape."

## *Conclusion and Future Directions*

Excitatory and inhibitory neurotransmitters exert a diversity of physiologic and pathologic effects, as determined by the type of ion channel directly coupled to (or ultimately modified by) the neurotransmitter receptor, the receptor location, the receptor subunit composition, and, sometimes, the interaction between multiple types of receptors (as in the cooperativity between AMPA and NMDA receptors). Because of the potential role of excitatory neurotransmission in a number of pathologic processes, such as hyperalgesia, stroke, trauma, and neurodegenerative diseases, glutamate receptors have become important targets for drug development. Barbiturates and benzodiazepines represent two classes of drugs used to amplify GABAergic transmission and, thereby, to achieve anxiolytic, anesthetic, sedative, hypnotic, or anticonvulsant effects. Inhibitory neurotransmission is also a path for future drug development as the understanding of the three classes of GABA receptors continues to progress. Ultimately, the growing knowledge of the physiologic and pathophysiologic function of excitatory and inhibitory neurotransmitter receptors will enable an expansion in the number and specificity of therapeutic agents that alter these targets.

## Suggested Readings

Ben-Ari Y, Cossart R. Kainate, a double agent that generates seizures: two decades of progress. Trends Neurosci 2000;23:580–587.

Hardingham GE, Bading H. The yin and yang of NMDA receptor signaling. Trends Neurosci 2003;26:81–89.

Hevers W, Lüddens H. Diversity of $GABA_A$ receptors. Mol Neurobiol 1998;18:35–86. (*A comprehensive review of $GABA_A$ receptor structure and function, with a focus on specific allosteric modulators (including benzodiazepines and barbiturates) and their effects on $Cl^-$ channel gating.*)

Kerr DIB, Ong J. $GABA_B$ receptors. Pharmacol Ther 1995;67:187–246. (*A detailed discussion of $GABA_B$ receptor structure, biochemistry, functions, and clinical correlations.*)

Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nature Rev Neurosci 2003;4:399–415.

Matute C, Alberdi E, Domercq M, Perez-Cerda F, et al. The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci 2001;24:224-230.

Olsen RW, Sapp DW, Bureau MH, Turner DM. Allosteric actions of central nervous system depressants including anesthetics on subtypes of the inhibitory γ-aminobutyric $acid_A$ receptor–chloride channel complex. Ann NY Acad Sci 1991;625:145–154.

Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. Prog Neurobiol 1998; 54: 581–618. (*A thorough review of ionotropic and metabotropic glutamate receptor physiology, pathophysiology, and pharmacology.*)

## Drug Summary Table Pharmacology of Excitatory and Inhibitory Neurotransmission

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF GLUTAMATE RELEASE AND GLUTAMATE RECEPTOR ANTAGONISTS**<br>*Mechanism—Although glutamate receptor pharmacology constitutes a growing field of development, many of the current drugs function by decreasing glutamate release rather than by directly modulating glutamate receptor function* | | | | |
| **Riluzole** | Prolongs survival in ALS because of its cerebroprotective, antiepileptic, and analgesic actions | Nausea, constipation, abdominal pain, increased ALT | | $Na^+$ channel blocker that inhibits presynaptic glutamate release |
| **Lamotrigine** | Adjunct therapy for complex partial seizures and seizures caused by ischemic cerebrovascular disease<br>Combined with carbidopa-levodopa for Parkinson's disease | Blurred vision, ataxia, nausea, headache, sedation, Stevens-Johnson syndrome | Induction of $P_{450}$ enzymes by other drugs (e.g., barbiturates) may increase metabolism | Prolongs inactivation period of $Na^+$ channels, thereby decreasing glutamate release (also, see Chapter 13) |
| **Tizanidine** | Skeletal muscle relaxant | Sedation, hypotension | | $\alpha_2$-Adrenergic agonist that decreases glutamate release and excitatory input to alpha motor neurons |
| **Amantadine** | Parkinson's disease (in combination with levodopa) | | | Noncompetitive blocker of NMDA channels |
| **Memantine** | Dementia associated with Alzheimer's disease<br>May slow neurodegeneration attributable to Parkinson's disease<br>Neuroleptic malignant syndrome | Does **not** cause the side affects associated with competitive NMDA antagonists (sedation, hypotension, ataxia, confusion, hallucinations, respiratory depression) | | Noncompetitive, use-dependent NMDA antagonist<br>Also blocks nACh receptors, $5\text{-}HT_3$ receptors |
| **BENZODIAZEPINES**<br>*Mechanism—Potentiate GABA actions by increasing its affinity for $GABA_A$ receptor* | | | | |
| **Clorazepate** | Generalized anxiety disorder, alcohol withdrawal, adjunct anticonvulsant for partial seizures | Sedation, ataxia, tremor, hypotension | Narrow angle glaucoma, pregnancy | Short-acting (3–8 hours) |
| **Alprazolam** | Anxiety disorders, phobias | Sedation, ataxia, tremor | Narrow angle glaucoma, pregnancy | Intermediate-acting (10–20 hours) |
| **Lorazepam** | Anxiety disorders, IV general anesthetic, status epilepticus | Sedation, ataxia, menstrual disorders, tremor | Narrow angle glaucoma, pregnancy | Intermediate-acting (10–20 hours) |
| **Chlordiazepoxide** | Anxiety disorders, acute alcohol withdrawal | Sedation, ataxia, menstrual disorders, tremor | Narrow angle glaucoma, pregnancy | Long-acting (1–3 days) |
| **Clonazepam** | Absence seizures, myoclonic seizures, panic disorder | Sedation, ataxia, menstrual disorders | Liver disease, narrow angle glaucoma, pregnancy | Long-acting (1–3 days) |
| **Diazepam** | Anxiety disorders, status epilepticus, muscle relaxant, IV general anesthetic, alcohol withdrawal | Sedation, ataxia, menstrual disorders, tremor | Narrow angle glaucoma, pregnancy | Long-acting (1–3 days) |

*(Continued)*

Drug Summary Table **Pharmacology of Excitatory and Inhibitory Neurotransmission** *(Continued)*

| | | | | |
|---|---|---|---|---|
| **BENZODIAZEPINES** *(Continued)*<br>*Mechanism—Potentiate GABA actions by increasing its affinity for GABA$_A$ receptor* | | | | |
| Triazolam | Short-term treatment of insomnia | Rebound insomnia, anterograde amnesia | Narrow angle glaucoma | Short-acting (3–8 hours) |
| Estazolam | Short-term treatment of insomnia | Rebound insomnia, muscle weakness | Narrow angle glaucoma, pregnancy | Intermediate-acting (10–20 hours) |
| Temazepam | Short-term treatment of insomnia | Sedation, euphoria, hangover, muscle weakness | Narrow angle glaucoma, pregnancy | Intermediate-acting (10–20 hours) |
| Flurazepam | Short-term treatment of insomnia | No rebound insomnia (less potent and slower elimination than shorter-acting benzodiazepines). Daytime sedation attributable to active metabolites. Palpitations, granulocytopenia, elevated AST/ALT, cholestatic jaundice | Narrow angle glaucoma, pregnancy | Long-acting (1–3 days) |
| Quazepam | Short-term treatment of insomnia | No rebound insomnia (less potent and slower elimination than shorter-acting benzodiazepines). Paranoid reaction, euphoria, nightmares, palpitations, daytime sedation | Narrow angle glaucoma, pregnancy | Long-acting (1–3 days) |
| Midazolam | Anxiolytic, preanesthetic, IV general anesthetic | Decreased respiratory rate | Narrow angle glaucoma, pregnancy | Short-acting (3–8 hours) |
| **GABA RECEPTOR ANTAGONISTS**<br>*Mechanism—Antagonist at GABA$_A$ receptor* | | | | |
| Flumazenil | Treatment/diagnosis of benzodiazepine overdose | | | Does not block effects of barbiturates or ethanol<br>Available only in intravenous form (extensive first-pass metabolism) |

**BARBITURATES**

*Mechanism—Potentiate GABA actions by increasing efficacy at the GABA$_A$ receptor. High doses of barbiturates can directly activate the GABA$_A$ receptor, giving them a narrower therapeutic window than benzodiazepines*

| | | | | |
|---|---|---|---|---|
| **Thiopental** | Anesthesia induction and short-term maintenance, increased intracranial pressure, emergency seizure treatment | Cardiovascular and respiratory depression, hallucinations, agranulocytosis, thrombocytopenia, megaloblastic anemia | Porphyria: barbiturates enhance porphyrin synthesis and exacerbate the disease, causing abdominal pain, seizures, hypertension, peripheral neuropathy (sensory and motor), respiratory failure, and death | Ultrashort-acting (5–15 minutes) |
| **Methohexital** | Anesthesia induction and short-term maintenance | Pain at injection site | Porphyria | Ultrashort-acting (5–15 minutes) |
| **Pentobarbital** | Insomnia, preoperative sedation, status epilepticus | Cardiovascular and respiratory depression, hallucinations, agranulocytosis, thrombocytopenia, megaloblastic anemia | Porphyria | Short-acting (3–8 hours) |
| **Secobarbital** | Insomnia, preoperative sedation | CNS depression, hangover effect | Porphyria | Short-acting (3–8 hours) |
| **Amobarbital** | Oral: short-term treatment of insomnia, preoperative sedation<br>IV, IM: status epilepticus, acute seizure treatment | CNS depression, hangover effect | Porphyria | Short-acting (3–8 hours) |
| **Phenobarbital** | Generalized tonic–clonic and partial seizures | CNS depression, hangover effect | Porphyria | Long-acting (days) |

# 11

# Pharmacology of Dopaminergic Neurotransmission

Scott M. Damrauer, Joshua M. Galanter, and David L. Cardozo

- Introduction
- Case
- Biochemistry and Cell Biology
  - Dopamine and the Catecholamine Neurotransmitters
  - Dopamine Storage, Release, Reuptake, and Inactivation
  - Dopamine Receptors
  - Dopamine Function
- Dopamine and Control of Movement: Parkinson's Disease
  - Physiology of Nigrostriatal Pathways
  - Pathophysiology
  - Pharmacologic Classes and Agents
    - *Dopamine Precursors*
    - *Dopamine Receptor Agonists*
    - *Inhibitors of Dopamine Metabolism*
    - *Nondopaminergic Pharmacology in Parkinson's Disease*
- Dopamine and Disorders of Thought: Schizophrenia
  - Pathophysiology
  - Pharmacologic Classes and Agents
    - *Mechanism of Action of Antipsychotic Drugs*
    - *Side Effects*
    - *Pharmacokinetics, Metabolism, and Drug Interactions*
    - *Atypical Antipsychotics*
- Conclusion and Future Directions
- Suggested Readings

## INTRODUCTION

As discussed in Chapter 8, Adrenergic Pharmacology, adrenergic neurons play a key role in the peripheral and central nervous systems. While peripheral adrenergic neurons and most central adrenergic neurons employ norepinephrine as the main transmitter, some central catecholamine-producing neurons lack the enzyme **dopamine β-hydroxylase** (the enzyme that catalyzes the formation of norepinephrine from dopamine). As a result, these neurons synthesize and use **dopamine (DA)** as their neurotransmitter. The machinery of dopaminergic and noradrenergic neurotransmission are similar in some components (such as biosynthetic and metabolic enzymes) but different in others (such as reuptake pumps and presynaptic and postsynaptic receptors). Ligand binding studies and dopamine receptor cloning have established that dopaminergic and noradrenergic receptors are distinct molecular entities. The existence of discrete dopaminergic pathways in the brain allows selective pharmacologic intervention in disorders of these pathways.

Dopaminergic pathway dysfunction results in several distinct pathologic states. This chapter presents the principles that underlie current therapies for diseases that directly or indirectly involve changes in DA neurotransmission. The chapter begins with a discussion of the biochemistry and cell biology of DA neurotransmission and the localization of the four major DA systems in the brain. Following this background, the chapter explores the physiology, pathophysiology, and pharmacology of **Parkinson's disease,** which results from the specific loss of neurons in one of these DA systems, and **schizophrenia,** which is currently treated with drugs that inhibit dopaminergic neurotransmission.

## ■ Case

Mark Smith is a 55-year-old man who goes to see his physician because he notices a tremor in his right hand. He is also having difficulty standing up from a sitting position and difficulty keeping his balance. His wife complains that he never smiles any more, and that his face is becoming expressionless. As Mr. Smith enters the examination room, his doctor notices that he is walking hunched over and has a short, shuffling gait. Mr. Smith reveals that he has stopped wearing dress shirts and lace-up shoes because of difficulty with the buttons and laces. The doctor finds on physical examination that Mr. Smith has increased tone and cogwheel rigidity in his upper extremities, that he is significantly slower than normal at performing rapid alternating movements, and that his balance while standing is reduced. The physician determines that Mr. Smith's symptoms and signs most likely represent the early stages of Parkinson's disease, and she prescribes a trial of levodopa.

## QUESTIONS

1. How does the loss of dopaminergic neurons result in symptoms such as those Mr. Smith is experiencing?
2. What will be the effect of levodopa on the course of Mr. Smith's disease?
3. How will Mr. Smith's response to his medication change over time?
4. What other types of therapies are available for Mr. Smith?

# BIOCHEMISTRY AND CELL BIOLOGY

## DOPAMINE AND THE CATECHOLAMINE NEUROTRANSMITTERS

**Dopamine** belongs to the **catecholamine** family of neurotransmitters. In addition to dopamine, this family includes **norepinephrine** (NE) and **epinephrine** (EPI). As the name suggests, the basic structure of the catecholamines consists of a catechol (3,4-dihydroxybenzene) moiety connected to an amine group by an ethyl bridge (Fig. 11-1A). Recall from Chapter 6, Principles of Nervous System Physiology and Pharmacology that catecholamines in the central nervous system (CNS) modulate the function of point-to-point neurotransmission and affect complex processes such as mood, attentiveness, and emotion. In the periphery, catecholamines are used as the major neurotransmitter by sympathetic postganglionic neurons and are secreted by the adrenal glands in response to stress.

The neutral amino acid **tyrosine** is the metabolic precursor for all catecholamines (Fig. 11-1B). The majority of tyrosine is obtained from the diet; a small proportion may also be synthesized in the liver from **phenylalanine.** The first step in the synthesis of DA is the conversion of tyrosine to **L-DOPA** (L-3,4-dihydroxyphenylalanine) by oxidation of the 3 position on the benzene ring. This reaction is catalyzed by the enzyme **tyrosine hydroxylase** (TH), a ferro (iron containing)-enzyme that consists of four identical subunits of approximately 60 kDa each. In addition to $Fe^{2+}$, TH also requires the cofactor tetrahydrobiopterin, which is oxidized to dihydrobiopterin in the course of the reaction. Importantly, oxidation of tyrosine to L-DOPA is the rate-limiting step in the production not only of DA, but of all catecholamine neurotransmitters.

The next and final step in the synthesis of DA is the conversion of L-DOPA to DA by the enzyme **aromatic amino acid decarboxylase (AADC,** sometimes called DOPA decarboxylase). AADC cleaves the carboxyl group from the α-carbon of the ethylamine side chain, liberating carbon dioxide. AADC requires the cofactor pyridoxal phosphate.

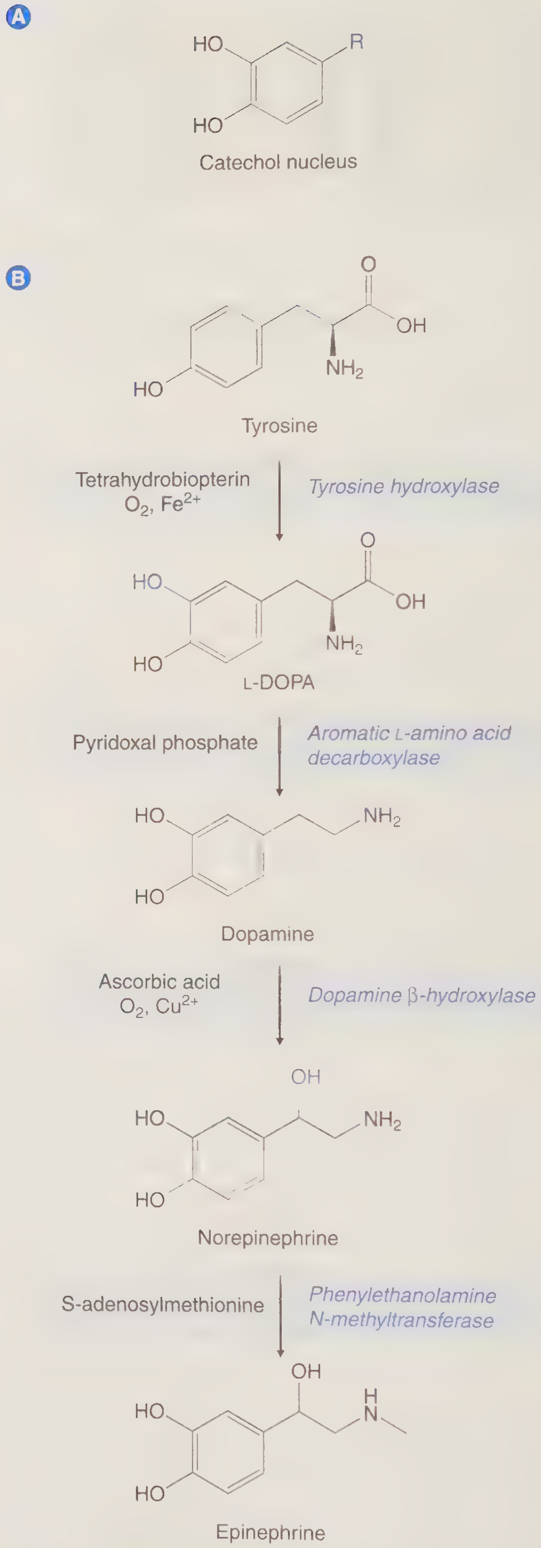


**Figure 11-1. Catecholamine Synthesis.** **A.** Catecholamines consist of a catechol nucleus with an ethylamine side chain (R group). The R group is ethylamine in dopamine, hydroxyethylamine in norepinephrine, and N-methyl hydroxyethylamine in epinephrine. **B.** Dopamine is synthesized from the amino acid tyrosine in a series of step-wise reactions. In cells that contain dopamine β-hydroxylase, dopamine can be further converted to norepinephrine; in cells that also contain phenylethanolamine N-methyltransferase, norepinephrine can be converted to epinephrine.

AADC is not specific for this reaction, however, because it is promiscuous in its ability to cleave carboxyl groups from the α-carbons of all aromatic amino acids. Furthermore, AADC expression is not restricted to the CNS; it is found throughout the body in almost all cell types.

In cells that secrete the catecholamine NE, DA is converted to NE by the enzyme **dopamine β-hydroxylase.** In other cells, NE may subsequently be converted to epinephrine by **phenylethanolamine N-methyltransferase.** It is important to keep in mind the entire pathway of catecholamine biosynthesis, because pharmacologic manipulation of DA biosynthesis can also alter the production of NE and EPI. For a more complete discussion of the last two steps in NE and EPI synthesis, see Chapter 8.

## DOPAMINE STORAGE, RELEASE, REUPTAKE, AND INACTIVATION

DA is synthesized from tyrosine in the cytoplasm of the neuron, then transported into secretory vesicles for storage and release (Fig. 11-2). Two separate molecular pumps are required for the transport of DA into synaptic vesicles. A proton ATPase concentrates protons in the vesicle, creating an electrochemical gradient characterized by a low intravesicular pH (i.e., a high proton concentration) and an electropositive vesicle interior. This gradient is exploited by a proton antiporter, which allows protons to move down the gradient (out of the vesicle) while simultaneously transporting DA into the vesicle against its concentration gradient. Upon nerve cell stimulation, the DA storage vesicles fuse with the plasma membrane in a $Ca^{2+}$-dependent manner, releasing DA into the synaptic cleft. In the cleft, DA can bind to both postsynaptic DA receptors and presynaptic DA autoreceptors (see below).

To prevent chronic overstimulation and eventual desensitization of DA receptors, several mechanisms exist to deplete synaptic DA. Most of the DA released into the synaptic cleft is transported back into the presynaptic cell by an 11-transmembrane domain protein that belongs to the family of catecholamine reuptake pumps. DA reuptake involves transport of the neurotransmitter against its concentration gradient, and, therefore, requires an energy source. For this reason, the DA reuptake transporter couples dopamine reuptake to the cotransport of $Na^+$ down its concentration gradient into the cell. In fact, both $Na^+$ and $Cl^-$ are cotransported with DA into the cell. Because the $Na^+$ gradient is maintained by the $Na^+/K^+$ ATPase pump, DA reuptake depends indirectly on the presence of a functioning $Na^+/K^+$ pump.

DA taken up into the presynaptic cell can either be recycled back into vesicles for further use in neurotransmission or degraded by the action of the enzyme **monoamine oxidase (MAO)** (Fig. 11-3). MAOs exist in two isoforms: MAO-A (which is found primarily in the periphery) and MAO-B (which functions in the CNS). Neither MAO-A nor MAO-B is specific for DA; both isoforms degrade a wide range of monoamine compounds. As noted in Chapter 8, gastrointestinal and hepatic MAO-A plays a key role in limiting the toxicity of the indirect-acting sympathomimetic **tyramine,** which is found in certain wines and cheeses.

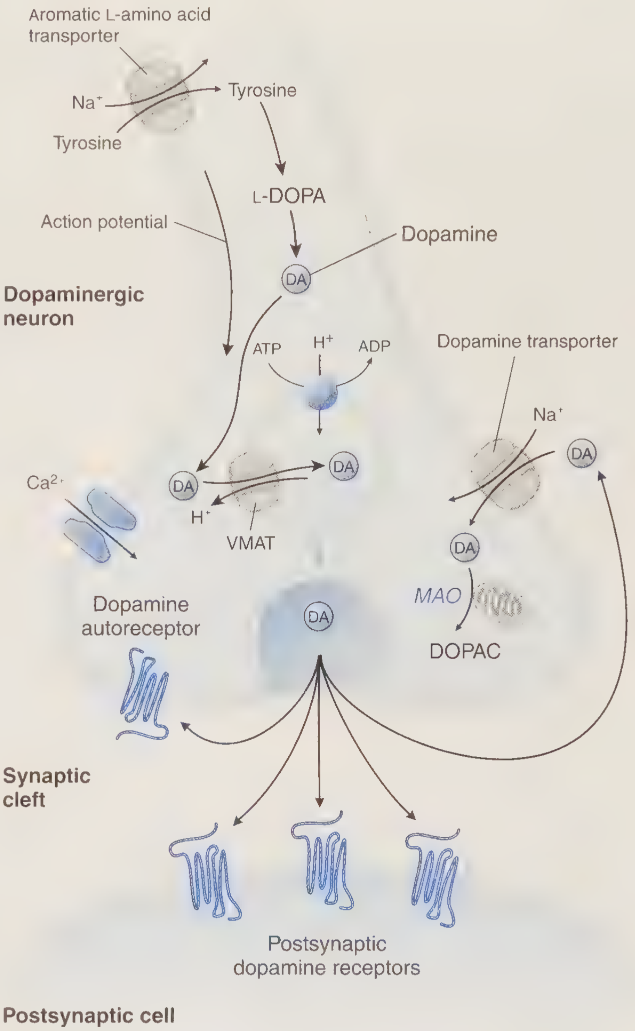


**Figure 11-2. Dopaminergic Neurotransmission.** Dopamine (DA) is synthesized in the cytoplasm and transported for storage in secretory vesicles by the action of a nonselective monoamine-proton antiporter (VMAT) that is powered by the electrochemical gradient created by a proton ATPase. Upon nerve cell stimulation, DA is released into the synaptic cleft, where the neurotransmitter can stimulate postsynaptic dopamine receptors and presynaptic dopamine autoreceptors. DA is transported out of the synaptic cleft by the selective, $Na^+$-coupled dopamine transporter. Cytoplasmic DA is retransported into secretory vesicles by VMAT or degraded by the enzyme monoamine oxidase (MAO).

Extracellular DA that is not taken up into the presynaptic cell can either diffuse out of the synaptic cleft or be degraded by the action of the enzyme **catechol-O-methyl transferase (COMT).** COMT is expressed in the brain, liver, kidney, and heart; it inactivates catecholamines by adding a methyl group to the hydroxyl group at the 3 position of the benzene ring. In the CNS, COMT is expressed primarily in postsynaptic neurons, although it is also expressed in certain presynaptic neurons. The sequential action of COMT and MAO degrades DA to the stable metabolite **homovanillic acid (HVA),** which is excreted in the urine (Fig. 11-3).

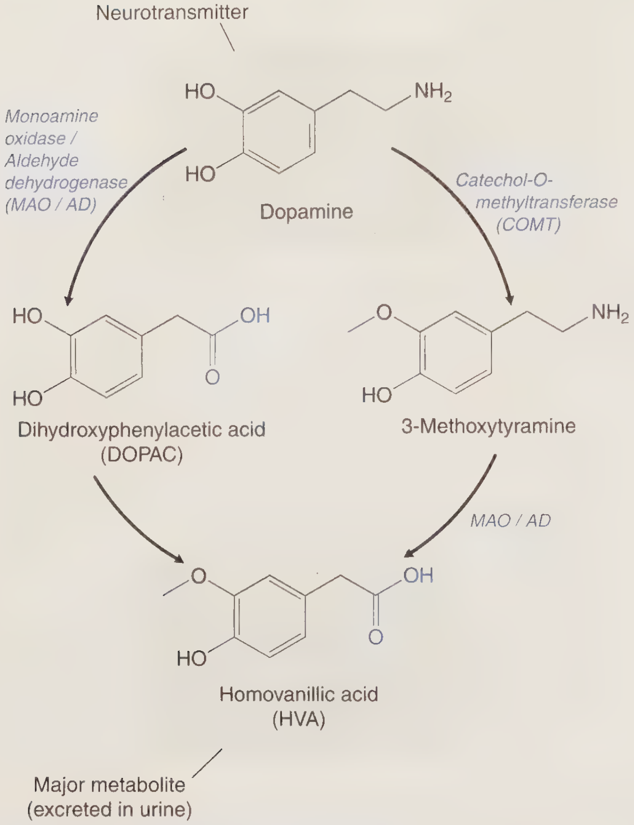

Neurotransmitter
HO
NH2
HO
Dopamine
Monoamine oxidase / Aldehyde dehydrogenase (MAO / AD)
Catechol-O-methyltransferase (COMT)
HO
OH
O
HO
Dihydroxyphenylacetic acid (DOPAC)
O
NH2
HO
3-Methoxytyramine
MAO / AD
O
OH
O
HO
Homovanillic acid (HVA)
Major metabolite (excreted in urine)

**Figure 11-3. Catecholamine Metabolism.** Dopamine is metabolized to homovanillic acid (HVA) in a series of reactions. Dopamine is oxidized to dihydroxyphenylacetic acid (DOPAC) by sequential action of the enzymes monoamine oxidase (MAO) and aldehyde dehydrogenase (AD). Catechol-O-methyl transferase (COMT) then oxidizes DOPAC to HVA. Alternatively, dopamine is methylated to 3-methoxytyramine by COMT, and then oxidized to HVA by MAO and AD. HVA, the most stable dopamine metabolite, is excreted in the urine.

## DOPAMINE RECEPTORS

Research in the 1970s first identified DA receptors based on their ability to bind radiolabeled DA and other known dopaminergic agonists. Further research revealed two main classes of dopamine receptors, with different affinities for a range of agonists and different effects on the production of intracellular cAMP. Currently, there are five known subtypes of DA receptors. Each subtype is encoded by a separate gene. All known DA receptors are seven transmembrane domain, G protein-coupled receptors. Many of these receptors exert their effects through the cAMP second messenger system. The five subtypes can be divided into two classes (Fig. 11-4): the **D1** class of receptors (D1 and D5) mediate an increase in cAMP, while the **D2** class ($D2_S$, $D2_L$, D3, and D4) cause a reduction in cAMP. The $D2_S$ (i.e., short) and $D2_L$ (i.e., long) receptors represent alternate splice variants of the same gene; their difference lies in the third cytoplasmic loop, which affects G protein interaction but not dopamine binding.

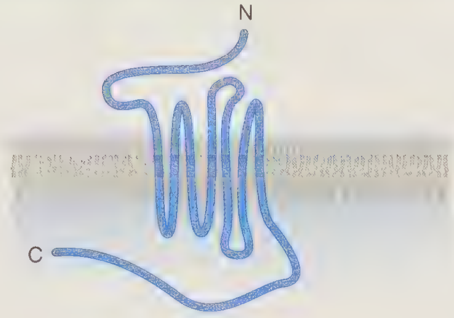
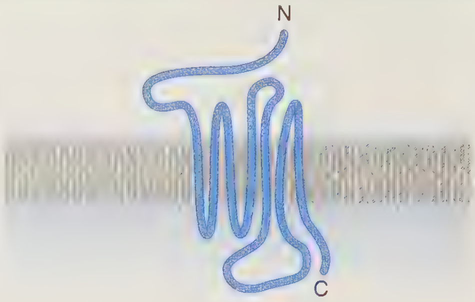
Autoradiography using radiolabeled agonists and antagonists has helped to elucidate the distribution of DA receptor subtypes in the CNS (Fig. 11-5). Although low levels of D1 and D2 receptor classes can be found throughout the brain, certain patterns of more specific localization have been observed. Both D1 and D2 receptors are expressed at high levels in the **striatum** (caudate and putamen), where they play a role in motor control by the **basal ganglia,** as well as in the **nucleus accumbens** (see Chapter 16, Pharmacology of Chemical Dependence and Addiction) and **olfactory tubercle.** D2 receptors are also expressed at high levels on anterior pituitary gland **lactotrophs,** where they regulate prolactin secretion (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). D2 receptors are thought to play a role in **schizophrenia** because many antipsychotic medications have high affinity for these receptors (see below), although the localization of the D2 receptors involved remains to be elucidated. D3 and D4 receptors are structurally and functionally related to D2 receptors, and may also be involved in the pathogenesis of schizophrenia. High levels of D3 receptors are found in the **limbic system,** including the nucleus accumbens and olfactory tubercle, while D4 receptors have been localized to the **frontal cortex, diencephalon,** and brainstem. D5 receptors are distributed sparsely and expressed at low levels, mainly in the **hippocampus** and **hypothalamus.** Interestingly, the affinity of D5 receptors for DA is 10-fold greater than that of D1 receptors for DA; the physiologic consequence of this finding is not yet known.

DA receptors can be found presynaptically on dopaminergic neurons as well as postsynaptically on target cells. Stimulation of presynaptic **DA autoreceptors** reduces dopaminergic tone, both by decreasing DA synthesis in the presynaptic neuron and by reducing the rate of neuronal firing. These autoreceptors, which belong to the D2 class of receptors, inhibit DA synthesis through down-regulation of cAMP-dependent TH activity. Their inhibitory effect on DA release and neuronal firing is due, in part, to a separate mechanism involving the modulation of $K^+$ and $Ca^{2+}$ channels. Increased $K^+$ channel opening results in a larger current that hyperpolarizes the neuron, so that a larger depolarization is needed to reach the firing threshold. Decreased $Ca^{2+}$ channel opening results in decreased levels of intracellular $Ca^{2+}$. Because $Ca^{2+}$ is required for synaptic vesicle trafficking to and fusion with the presynaptic membrane, decreases in intracellular $Ca^{2+}$ levels result in decreased dopamine release.

## DOPAMINE FUNCTION

Most central dopaminergic neurons originate in discrete areas of the brain, as shown in Figure 11-6 (see also Fig. 6-8). Definitive identification of these areas was achieved in the 1950s and 1960s by using **histofluorescence** techniques. When added to a tissue sample, aldehydes react with the catecholamines present in the cells to form a fluorescent adduct that can be detected in situ using fluorescence microscopy. The development of this technique was a great advance over previous methods because it preserves cellular morphology and leaves individual cells intact.

Using histofluorescence, neuroanatomists have identified four areas in the brain that contain particularly high concentrations of DA. The major DA tract is the **nigrostriatal** system, which contains about 80% of the brain's DA. This tract projects rostrally from cell bodies in the **pars compacta** of

| | **D1 Receptor Family** | **D2 Receptor Family** |
|---|---|---|
| Schematic structure |  |  |
| Second messenger systems | ↑ cAMP (via $G_s$)<br>↑ $PIP_2$ hydrolysis<br>$Ca^{2+}$ mobilization (via $IP_3$)<br>PKC activation | ↓ cAMP (via $G_i$)<br>↑ $K^+$ currents<br>↓ Voltage-gated $Ca^{2+}$ currents |

| | **D1** | **D5** | **D2** | **D3** | **D4** |
|---|---|---|---|---|---|
| Distribution in CNS | Striatum<br>Neocortex | Hippocampus<br>Hypothalamus | Striatum<br>Substantia nigra<br>Pituitary gland | Olfactory tubercle<br>Nucleus accumbens<br>Hypothalamus | Frontal cortex<br>Medulla<br>Midbrain |

**Figure 11-4.** **Dopamine Receptor Families.** The five dopamine receptor subtypes (D1–D5) can be classified into two major families of receptors. The D1 receptor family has a short 5–6 cytoplasmic loop and a long C-terminal tail; whereas, the D2 receptor family has a long 5–6 cytoplasmic loop and a short C-terminal tail. Stimulation of the D1 family is excitatory, increasing cAMP and intracellular $Ca^{2+}$ levels and activating protein kinase C (PKC). Stimulation of the D2 family is inhibitory, decreasing cAMP and intracellular $Ca^{2+}$ levels and hyperpolarizing the cell. The five receptor subtypes exhibit distinctive patterns of distribution in the central nervous system. Within the D2 receptor subtype, there are $D2_S$ and $D2_L$ isoforms.

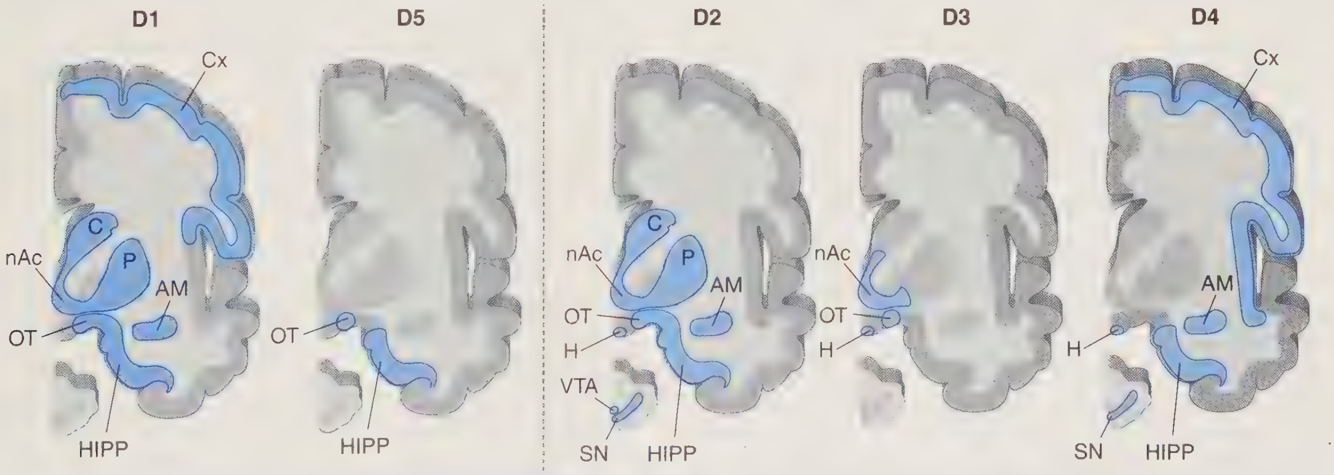


**Figure 11-5.** **Location of Dopamine Receptors in the Brain.** The location of the five dopamine receptor subtypes in the human brain, as determined by localization of receptor mRNAs in corresponding regions of the rat brain, is shown in blue in coronal section. Both D1 and D2 receptors are localized in the caudate and putamen (the striatum), nucleus accumbens, amygdala, olfactory tubercle, and hippocampus. In addition, D1 receptors are present in the cerebral cortex; whereas, D2 receptors are present in the substantia nigra, ventral tegmental area, and hypothalamus. Abbreviations: Cx = cerebral cortex, C = caudate, P = putamen, nAc = nucleus accumbens, AM = amygdala, OT = olfactory tubercle, HIPP = hippocampus, H = hypothalamus, SN = substantia nigra, VTA = ventral tegmental area.

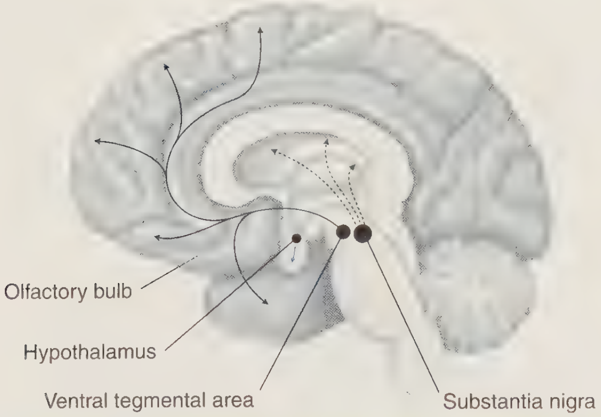


**Figure 11-6. Dopaminergic Nuclei and their Projections in the CNS.** Dopaminergic neurons originate in a number of specific nuclei in the brain. Neurons that originate in the hypothalamus and project to the pituitary gland (blue arrow) are tonically active and inhibit prolactin secretion. Neurons that project from the substantia nigra to the striatum regulate movement. Dopaminergic neurons that project from the ventral tegmental area to the limbic system and prefrontal cortex are thought to have roles in the regulation of mood and behavior.

the **substantia nigra** to terminals that richly innervate the striatum. The striatum is named for the striped appearance of the white fiber tracts that run through it; the substantia nigra is named for the dark pigmentation that results from the decomposition of DA to melanin. Dopaminergic neurons of the nigrostriatal system are involved in the stimulation of purposeful movement. Their degeneration results in Parkinson's disease, as discussed below.

Medial to the substantia nigra is an area of dopaminergic cell bodies in the midbrain called the **ventral tegmental area (VTA).** The VTA innervates several forebrain areas, most notably the cerebral cortex and limbic structures. These systems play an important and complex (yet poorly understood) role in motivation, goal-directed thinking, regulation of affect, and positive reinforcement (reward). Derangement of these pathways may be involved in the development of **schizophrenia;** as discussed below, the blocking of dopaminergic neurotransmission can lead to a remission in psychotic symptoms. (See Chapter 16 for a more complete discussion of the reward pathway.)

DA-containing cell bodies in the **arcuate** and **periventricular nuclei** of the hypothalamus project axons to the median eminence of the hypothalamus and to the intermediate lobe of the pituitary gland. This system is known as the **tubero-infundibular** pathway. The release of DA from these neurons tonically inhibits the release of prolactin by pituitary lactotrophs.

Finally, the **area postrema,** located in the wall of the caudal end of the **fourth ventricle,** contains a high concentration of DA receptors. The area postrema is one of the **circumventricular organs** that function as blood chemoreceptors through a local breach in the blood–brain barrier. Stimulation of DA receptors in the area postrema activates the vomiting centers of the brain and is one of the causes of **emesis.**

A derangement in any of these dopaminergic systems can result in disease. Parkinson's disease, which arises from dysregulated dopamine neurotransmission, and schizophrenia, which may also result from abnormal dopamine neurotransmission, are two such examples. These two diseases, and the pharmacologic interventions used to treat them, are highlighted below. Because the pharmacologic manipulation of dopaminergic systems is not always specific to one system, many of the side effects of drugs that act on these systems can be predicted based on their effects on the other dopaminergic systems.

## DOPAMINE AND CONTROL OF MOVEMENT: PARKINSON'S DISEASE

### PHYSIOLOGY OF NIGROSTRIATAL PATHWAYS

As noted above, the nigrostriatal pathway is an important regulator of purposeful movement. In more detail, the striatum is the origin of a system of pathways, termed the **direct pathway** and **indirect pathway,** which are modulated by dopamine release from nigrostriatal neurons (Fig. 11-7). These two pathways act in concert with one another to ensure that purposeful movement is initiated yet controlled. The direct pathway involves GABAergic neurons from the striatum that inhibit GABAergic neurons from the internal segment of the **globus pallidus.** The latter neurons tonically inhibit the thalamus, which in turn, sends excitatory projections to the cortex that initiate movement. In this manner, activation of the direct pathway disinhibits the thalamus; that is, the *direct pathway stimulates movement.*

Like the direct pathway, the indirect pathway involves GABAergic neurons from the striatum. This pathway inhibits neurons in the external segment of the globus pallidus, which in turn, inhibits neurons in the **subthalamic nucleus.** The neurons in the subthalamic nucleus are excitatory glutamatergic neurons that project to the internal segment of the globus pallidus. As with direct pathway neurons in the internal segment, the indirect pathway GABAergic neurons inhibit the thalamus. However, unlike direct pathway neurons, the indirect pathway neurons are generally not active at baseline. Thus, activation of the indirect pathway disinhibits neurons of the subthalamic nuclei that, in turn, stimulate neurons in the internal segment of the globus pallidus to inhibit the thalamus; that is, *the indirect pathway inhibits movement.*

DA from the nigrostriatal pathway stimulates neurons of the direct pathway and inhibits neurons of the indirect pathway. Notice that both of these effects promote movement. The stimulatory effects of DA on the direct pathway are mediated by D1 receptors, while the inhibitory effects of DA on the indirect pathway are mediated by D2 receptors.

### PATHOPHYSIOLOGY

In Parkinson's disease, there is selective loss of dopaminergic neurons in the **substantia nigra pars compacta.** Because these dopaminergic neurons regulate the function of the basal ganglia, which are important in the initiation and control of movement, the destruction of these neurons results in generalized **hypokinesia,** or lack of movement (Fig. 11-8). Specifically, patients with Parkinson's disease have difficulty

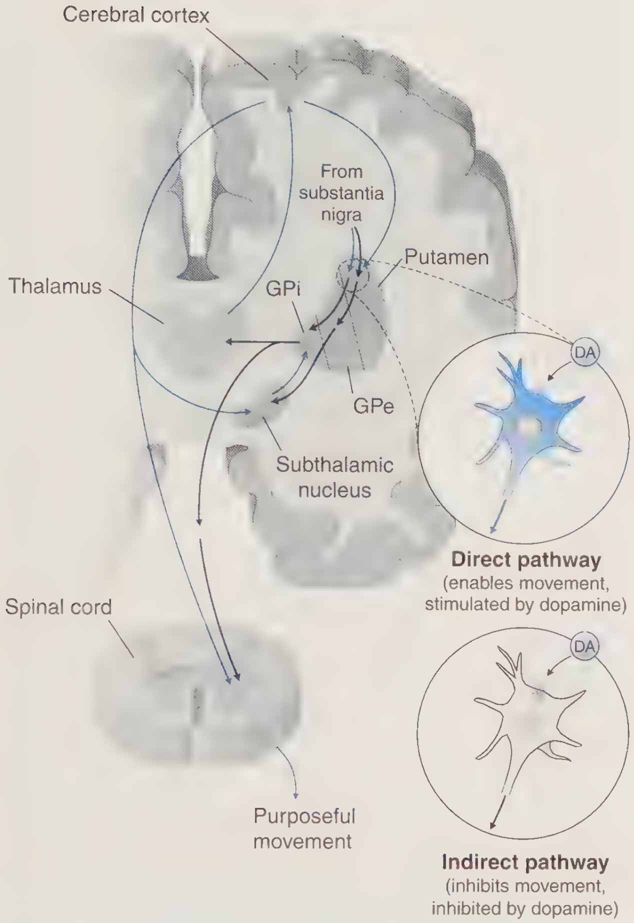

Cerebral cortex
From substantia nigra
Putamen
Thalamus
GPi
DA
GPe
Subthalamic nucleus
Direct pathway
(enables movement, stimulated by dopamine)
Spinal cord
DA
Purposeful movement
Indirect pathway
(inhibits movement, inhibited by dopamine)

**Figure 11-7. Dopaminergic Pathways for the Regulation of Movement.** There are two principal pathways in the basal ganglia that regulate movement: the indirect pathway, which inhibits movement, and the direct pathway, which promotes movement. Both are affected by dopamine signaling from the substantia nigra. Dopamine inhibits the indirect pathway and stimulates the direct pathway, yielding a net bias that allows purposeful movement. Excitatory pathways are shown in blue, and inhibitory pathways are shown in black. Note that the direct pathway signals from putamen to GPi to thalamus to cortex, while the indirect pathway signals from putamen to GPe to subthalamic nucleus to GPi to thalamus to cortex. GPi = internal segment of the globus pallidus; Gpe = external segment of the globus pallidus.

initiating and maintaining voluntary muscle movement, and display a characteristic muscle rigidity and tremor.

In all but a few specific types of Parkinson's disease, the mechanism underlying the destruction of DA neurons in the substantia nigra is not fully understood. In 1983, the unexpected development of Parkinson's disease in abusers of the synthetic opioid meperidine (see Chapter 15, Pharmacology of Analgesia) yielded the first agent known to produce Parkinson's disease directly, and the first animal model of the disease. These abusers, who tended to be young and otherwise healthy, suddenly developed severe, levodopa-responsive parkinsonian symptoms. The abusers were all linked to a single contaminated batch of meperidine that had been synthesized in a makeshift lab. The contaminant was found to be MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which forms as an impurity in the synthesis of meperidine when its manufacture is carried out for too long and at too high a temperature. Studies in nonhuman primates have shown that MPTP is oxidized in the brain to MPP$^+$ (1-methyl-4-phenyl-pyridinium), which is selectively toxic to neurons in the substantia nigra.

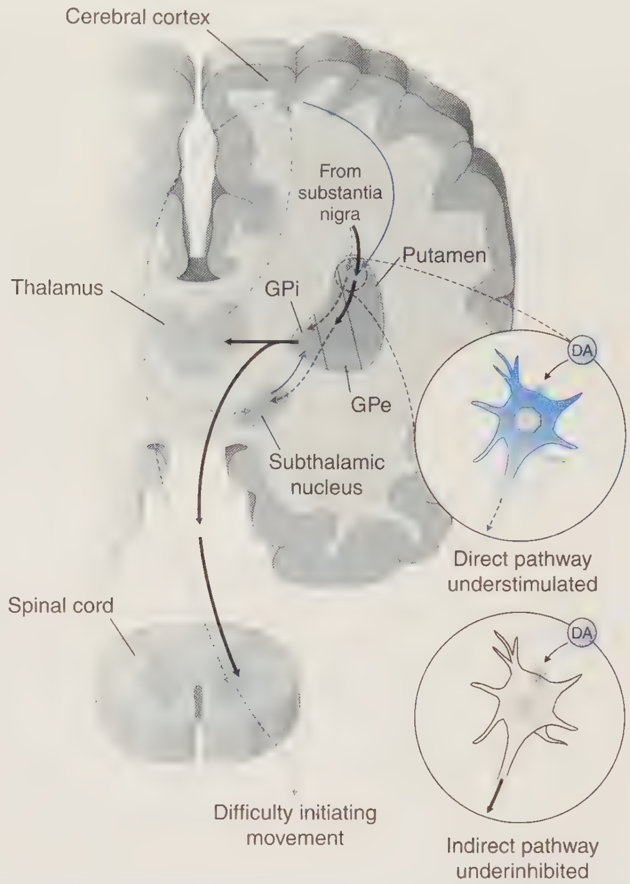

Cerebral cortex
From substantia nigra
Putamen
Thalamus
GPi
DA
GPe
Subthalamic nucleus
Direct pathway understimulated
Spinal cord
DA
Difficulty initiating movement
Indirect pathway underinhibited

**Figure 11-8. Effect of Parkinson's Disease on the Dopaminergic Pathways that Regulate Movement.** Degeneration of dopaminergic neurons in the substantia nigra results in understimulation of the direct (movement-enabling) pathway and underinhibition of the indirect (movement-inhibiting) pathway. The net result is a paucity of movement. Excitatory pathways are shown in blue, and inhibitory pathways are shown in black. Dotted lines indicate decreased activity caused by understimulation or overinhibition, and thicker lines indicate increased activity caused by overstimulation or underinhibition. GPi = internal segment of the globus pallidus; GPe = external segment of the globus pallidus.

Other than the rare patient who has Parkinson's disease secondary to MPTP exposure, however, the etiology of Parkinson's disease in most patients is unknown. Some cases are associated with genetic mutations, such as an autosomal recessive mutation in the parkin gene on chromosome 6q25.2-27, or an autosomal dominant mutation in the α-synuclein gene on chromosome 4q21.23. Although the discovery of these mutations has allowed the development of transgenic mouse and fruit fly models, most cases of Parkinson's disease are not associated with known genetic mutations. Despite the lack of linkage to a known mutation, there is an increased predisposition to the disease among family members of Parkinson's disease patients. The etiology of Parkinson's disease in most patients will likely turn out to be multifactorial, with contributions from both genetic and environmental factors.

## PHARMACOLOGIC CLASSES AND AGENTS

Because the destruction of DA neurons in the nigrostriatal system is irreversible, symptom management is the current standard of treatment for Parkinson's disease (PD). Pharmacologic intervention is aimed at restoring DA levels in the brain, thereby ameliorating symptoms that interfere with the patient's activities of daily life. In general, medications used in the management of PD can be divided into DA precursors, DA receptor agonists, and inhibitors of DA degradation. Although these compounds are useful in reducing the severity and frequency of symptoms, especially early in the course of the disease, they do not delay the progression of the illness. Significant current research is directed at developing neuroprotective compounds that slow the progression of PD and/or restore normal dopaminergic function; no current therapy accomplishes these tasks, however.

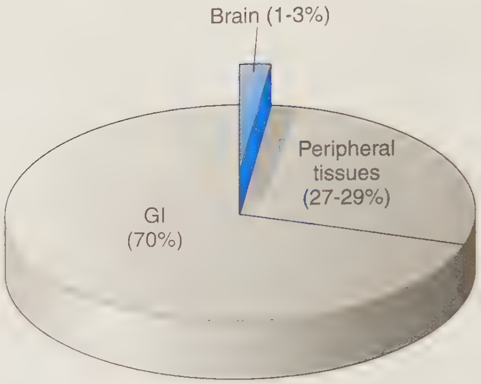
### Dopamine Precursors

**Levodopa** was first used to treat PD over 30 years ago, and is still the most effective treatment for the disease. DA itself is not suitable for the treatment of PD because it cannot cross the **blood–brain barrier** (BBB). However, DA's immediate precursor, L-DOPA (levodopa), is readily carried across the BBB by the neutral amino acid transporter (see Chapter 6); once in the CNS, L-DOPA is converted to dopamine. Thus, L-DOPA must compete with other neutral amino acids for transport across the BBB, and its availability in the CNS may be compromised by recent protein meals (see the introductory case in Chapter 6).

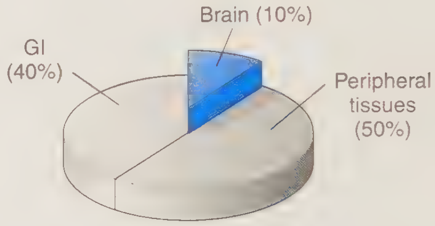
Orally administered levodopa is readily metabolized by decarboxylases and by MAO-A in the gastrointestinal tract, before it can enter the CNS; these inactive metabolites are then excreted in the urine. In addition, DOPA decarboxylase in the periphery converts much of the remaining drug into dopamine before it can cross the BBB. These processes both diminish the amount of levodopa that can reach the brain, and increase the peripheral side effects (predominantly nausea) that result from the generation of dopamine in the peripheral circulation. Under these conditions, only 1%–3% of the administered dose is available in the CNS (Fig. 11-9).

To boost the levels of levodopa available to the brain and to reduce the side effects of peripheral levodopa metabolism, levodopa is almost always administered in combination with **carbidopa,** an inhibitor of DOPA decarboxylase. Carbidopa effectively prevents the conversion of levodopa to DA in the periphery. Importantly, carbidopa is not able to cross the BBB and, therefore, does not interfere with the conversion of levodopa to DA in the CNS. Thus, carbidopa increases the fraction of orally administered levodopa available in the CNS from 1%–3% to 10%, allowing a significant reduction in the dose of levodopa and reducing the incidence of peripheral side effects.

Many patients with PD show remarkable symptomatic improvement on the levodopa/carbidopa combination, especially during the early phase of the disease. In fact, an improvement in symptoms following the initiation of levodopa therapy is considered diagnostic of PD. Over time, however, the effectiveness of levodopa declines. Continued use results in both tolerance and sensitization to the medication, manifested as a drastic narrowing of the therapeutic window. As patients continue on levodopa therapy, they require more drug to produce a clinically significant improvement in symptoms. They develop fluctuations in motor function that include periods of freezing and increased rigidity, known as "off" periods, alternating with periods of normal or even dyskinetic movement, known as "on" periods. These "on" periods generally occur shortly after the administration of levodopa/carbidopa, when a large bolus of dopamine is delivered to the striatum. "On" periods can be overcome initially by taking smaller doses of medication, although this increases the likelihood of "off" periods. "Off" periods tend to occur as plasma levels of levodopa decline and can be compensated for by increasing either the dose of levodopa or the frequency of dosing of the medication. At least initially, dosing within the narrower therapeutic window can be achieved by administering smaller doses of levodopa more frequently, or by using extended release formulations of the drug.



Dose of levodopa (single agent)



Dose of levodopa (in combination with carbidopa)

**Figure 11-9. Effects of Carbidopa on the Peripheral Metabolism of Levodopa.** Orally administered levodopa is metabolized in the peripheral tissues and in the gastrointestinal (GI) tract by aromatic L-amino acid decarboxylase (AADC) and by monoamine oxidase (MAO). This metabolism significantly reduces the effective dose available to the brain and significantly increases the peripheral side effects of levodopa. Carbidopa is an AADC inhibitor that cannot cross the blood–brain barrier. When levodopa is administered in combination with carbidopa, a greater fraction of the levodopa is available to the brain. Therefore, a smaller dose of levodopa is required for clinical efficacy. Thus, although the fraction of levodopa that remains in peripheral tissues increases when levodopa is administered in combination with carbidopa, the total amount of levodopa that remains in peripheral tissues decreases, and fewer peripheral side effects occur. In this figure, the volume of each pie slice denotes the absolute amount of levodopa that is administered or metabolized, or that remains in peripheral tissues or crosses the blood–brain barrier.

The most profound side effect of levodopa is its propensity to cause **dyskinesias,** or uncontrollable rhythmic movements of the head and trunk. These movements are nearly ubiquitous in patients taking levodopa, and they generally worsen as the disease progresses. Similar to the "on/off" phenomenon, dyskinesias are initially linked to levodopa dosing, occurring at times of maximal levodopa plasma concentrations. Accordingly, dyskinesias can also be managed initially by using smaller doses of levodopa more frequently. Unfortunately, as the disease progresses, continued therapy leads to worsening of both the dyskinesias and the "on/off" phenomenon, to the point where one or the other is almost always present.

Although dyskinesias and fluctuations in motor function are complex, poorly understood side effects of therapy, at least two factors are thought to contribute to these effects. First, the continued destruction of dopaminergic neurons as Parkinson's disease progresses results in the striatum's increasing inability to store dopamine effectively. Shortly after the administration of an oral dose of levodopa, a bolus of the drug is delivered to a small number of dopaminergic neurons in the striatum, and these neurons convert the levodopa to dopamine. The immediate release of neurotransmitter into the synaptic cleft results in an "on" period and in dyskinesias. Because there are few dopaminergic neurons left, however, they are unable to store the dopamine effectively. As the plasma (and therefore the brain) concentration of levodopa falls, the neurons convert less and less levodopa to dopamine. Neuronal dopamine stores are quickly depleted, resulting in the release of insufficient dopamine into the synaptic cleft and an "off" period. Second, chronic therapy with levodopa seems to cause adaptations in postsynaptic neurons in the striatum. Fluctuations in the concentrations of dopamine to which postsynaptic neurons are exposed induce changes in the cell surface expression of dopamine receptors and in postreceptor signaling events. These postsynaptic adaptations alter the cell's sensitivity to synaptic dopamine levels, further accentuating responses associated with high ("on" period, dyskinesia) and low ("off" period, akinesia) transmitter concentrations.

The predictable decline in efficacy and increase in side effects that result from prolonged levodopa therapy have led to discussions about the appropriate time to begin treatment of PD with levodopa and the relative merits of delaying the use of this drug in the early stages of the disease. It is generally accepted that levodopa therapy should be initiated as soon as other therapies (see below) are unable to control Parkinsonian symptoms effectively. Studies have shown that further delays in levodopa therapy are associated with reduced rates of symptom control and increased mortality.

## Dopamine Receptor Agonists

Another strategy for enhancing dopaminergic neurotransmission is to target the postsynaptic DA receptor directly through the use of DA receptor agonists. Ergot derivatives, such as **bromocriptine** (D2 agonist) and **pergolide** (D1 and D2), and nonergot agonists, such as **pramipexole** (D3>D2) and **ropinirole** (D3>D2), have all been used successfully as adjuvants to levodopa treatment. Some studies have suggested that initial monotherapy with DA agonists, and early combined treatment with DA agonists after the initiation of levodopa/carbidopa therapy, may delay the onset of motor fluctuations in PD and prolong the period of time over which levodopa/carbidopa therapy is effective, although these findings remain speculative. In vitro and neuroimaging studies support the hypothesis that these benefits may result from a neuroprotective action of DA agonists.

As a class, DA receptor agonists have several advantages. Because they are nonpeptide molecules, they do not compete with levodopa or other neutral amino acids for transport across the BBB. Furthermore, they do not require enzymatic conversion by DOPA decarboxylase to be effective, and they could therefore remain effective late in the course of PD. Bromocriptine, pergolide, pramipexole, and ropinirole all exhibit differential binding to subclasses of DA receptors, and they vary in their pharmacologic profiles and side effects. The agonists that have a high affinity for D2 and D3 receptors can potentially cause psychotic side effects (see below), although they tend to have fewer peripheral side effects than levodopa.

## Inhibitors of Dopamine Metabolism

A third strategy that has been successfully employed to treat PD involves the inhibition of DA breakdown. Inhibitors of both MAO-B (the isoform of MAO that predominates in the striatum) and COMT have been used as adjuvants to levodopa in clinical practice. **Selegiline** is a MAO inhibitor that, in low doses, is selective for MAO-B. It does not, therefore, interfere with the peripheral metabolism of monoamines by MAO-A. Consequently, selegiline avoids the toxic effects of dietary tyramine that are associated with nonselective MAO blockade (see Chapter 12, Pharmacology of Affective Disorders). Selegiline use is associated with enhanced motor function in patients who are simultaneously taking levodopa.

**Tolcapone** and **entacapone** inhibit the degradation of DA by inhibiting COMT. Tolcapone is a highly lipid soluble agent that can cross the BBB, while entacapone distributes only to the periphery. The functional result of tolcapone therapy is similar to that of selegiline, because both agents reduce the breakdown of DA in the CNS. Entacapone, on the other hand, produces a result similar to that of carbidopa because it decreases the peripheral metabolism of levodopa and, thereby, makes more levodopa available to the CNS. Because entacapone and carbidopa work by different mechanisms, entacapone can be used to augment the effect of carbidopa on the bioavailability of levodopa. In clinical trials, these COMT inhibitors have been shown to reduce the "off" periods that are associated with decreasing plasma levodopa levels (see above).

## Nondopaminergic Pharmacology in Parkinson's Disease

**Amantadine, trihexyphenidyl,** and **benztropine** are all drugs that do not affect dopaminergic pathways directly but are nonetheless effective in the treatment of PD. **Amantadine** was developed and is marketed primarily as an antiviral that reduces the length and severity of influenza A infections (see Chapter 34, Pharmacology of Viral Replication). In patients with PD, however, amantadine is used to treat levodopa-induced dyskinesias that develop late in the course of the disease. The mechanism by which amantadine reduces dyskinesia is thought to involve global blockade of excitatory NMDA receptors. **Trihexyphenidyl** and **benztropine** are both muscarinic receptor antagonists that reduce cholinergic tone in the CNS. They reduce tremor more than bradykinesia, and are, therefore, more effective in treating patients for whom tremor is the major clinical manifestation of PD.

## DOPAMINE AND DISORDERS OF THOUGHT: SCHIZOPHRENIA

### PATHOPHYSIOLOGY

Schizophrenia is a thought disorder characterized by one or more episodes of psychosis (impairment in reality testing). Patients may manifest disorders of perception, thinking, speech, emotion, and/or physical activity. Schizophrenic symptoms have been divided into two broad categories. **Positive symptoms** involve the development of abnormal functions; these symptoms include **delusions** (distorted or false beliefs and misinterpretation of perceptions), **hallucinations** (abnormal perceptions, especially auditory), **disorganized speech,** and **catatonic behavior. Negative symptoms** involve the reduction or loss of normal functions; these symptoms include **affective flattening** (decrease in the range or intensity of emotional expression), alogia (decrease in the fluency of speech), and **avolition** (decrease in the initiation of goal-directed behavior). The American Psychiatric Association criteria for making a diagnosis of schizophrenia are listed in Box 11-1.

Schizophrenia typically affects individuals in their late teens and early 20s. The disorder affects males and females equally. Approximately 4.75 million individuals suffer from schizophrenia in the United States, and 100,000 to 150,000 new cases are diagnosed annually. A genetic component of the disease has been demonstrated, but concordance among identical twins is only 50%. Schizophrenia, therefore, seems to have a multifactorial etiology, with both genetic and environmental components.

Although many theories exist, the pathogenesis and pathophysiology of schizophrenia are not understood at a fundamental level. Importantly, treatment with DA receptor antagonists, specifically D2 antagonists, relieves a number of the symptoms of schizophrenia in many, but not all, patients with the disease. The use of DA receptor antagonists to treat schizophrenia is based solely on their clinical success in relieving symptoms and improving quality of life. Although this therapeutic success has led to significant speculation as

BOX 11-1. Criteria for Schizophrenia, from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):
   1. Delusions
   2. Hallucinations
   3. Disorganized speech (e.g., frequent derailment or incoherence)
   4. Grossly disorganized or catatonic behavior
   5. Negative symptoms (i.e., affective flattening, alogia, or avolition)

   *Note:* Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts, or two or more voices conversing with each other.

B. Social/occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved before the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and mood disorder exclusion: Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either (1) no major depressive, manic, or mixed episodes have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not attributable to the direct physiologic effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a pervasive developmental disorder: If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least 1 month (or less if successfully treated).

---

Classification of longitudinal course (can be applied only after at least 1 year has elapsed since the initial onset of active-phase symptoms):
   Episodic with interepisode residual symptoms (episodes are defined by the reemergence of prominent psychotic symptoms); also specify if with prominent negative symptoms
   Episodic with no interepisode residual symptoms
   Continuous (prominent psychotic symptoms are present throughout the period of observation); also specify if with prominent negative symptoms
   Single episode in partial remission; also specify if with prominent negative symptoms
   Single episode in full remission

to the causes of schizophrenia and its underlying pathophysiology, the basis for the efficacy of antipsychotic medications in treating the disease remain to be elucidated.

The theory that is most commonly cited to explain the pathogenesis of schizophrenia is the **dopamine hypothesis,** which states that the illness is caused by increased and dysregulated levels of DA neurotransmission in the brain. Although the DA hypothesis is based primarily on the therapeutic effectiveness of DA receptor antagonists in treating schizophrenia, it is supported by several additional clinical observations. First, some patients taking drugs that increase DA levels or that activate dopamine receptors in the CNS, including **amphetamines, cocaine,** and **apomorphine,** develop a schizophrenia-like state that subsides when the dose of the drug is lowered. Second, hallucinations are a known side effect of levodopa therapy for Parkinson's disease. Finally, because treatment with DA receptor-blocking antipsychotics changes the levels of the DA metabolite HVA in the plasma, urine, and CSF, researchers have been able to correlate decreased DA metabolite levels, and by extension decreased DA levels, with clinical improvement in schizophrenic symptoms.

The dysregulation of DA neurotransmission in schizophrenia is thought to occur at specific anatomical locations in the brain. The **mesolimbic system** is a dopaminergic tract that originates in the ventral tegmental area and projects to the nucleus accumbens, ventral striatum, parts of the amygdala and hippocampus, and other components of the limbic system. This system is involved in the development of emotions and memory, and some hypothesize that mesolimbic hyperactivity is responsible for the positive symptoms of schizophrenia. This hypothesis is supported by positron emission tomography (PET) scans of the brains of patients displaying the earliest signs of schizophrenia; these PET images show changes in blood flow to the mesolimbic system that represent changes in the level of functioning of this system.

Dopaminergic neurons of the **mesocortical system** originate in the ventral tegmental area and project to regions of the cerebral cortex, particularly the prefrontal cortex. Because the prefrontal cortex is responsible for attention, planning, and motivated behavior, the hypothesis has been advanced that the mesocortical system plays a role in the negative symptoms of schizophrenia. It is unknown for certain, however, whether hyperactivity or hypoactivity of dopaminergic neurons is responsible for the positive and negative manifestations of the disease.

One hypothesis suggests that schizophrenia is caused by an imbalance between the mesolimbic and mesocortical systems, and specifically, by a decrease in mesocortical activity and a concomitant increase (potentially compensatory) in mesolimbic activity. All of the evidence implicating DA in the pathogenesis of schizophrenia is circumstantial, however, and much of it is conflicting. It should be noted that changes in DA levels, particularly in the mesolimbic and mesocortical systems, could simply reflect downstream consequences of a pathologic process in a heretofore undiscovered pathway.

One theory involving such an upstream process suggests that an imbalance in glutamatergic neurotransmission (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission) plays an important role in schizophrenia. This theory is supported by the observation that the drug phencyclidine (PCP) (see Chapter 16, Pharmacology of Chemical Dependence and Addiction), an antagonist at NMDA receptors, causes symptoms similar to those of schizophrenia. In fact, the syndrome seen in patients taking PCP chronically, which consists of psychotic symptoms, visual and auditory hallucinations, disorganized thought, blunted affect, withdrawal, psychomotor retardation, and an amotivational state, has components of both the positive and negative symptoms of schizophrenia. Because dopaminergic neurons and excitatory glutamatergic neurons often form reciprocal synaptic connections, the efficacy of DA receptor antagonists in schizophrenia could be based on the ability of these drugs to alleviate the disease's symptoms without treating its underlying cause (hypothesized here to involve dysregulated glutamatergic neurotransmisson). Even if this were the case, it is important to note that glutamate receptors are ubiquitous and involved generally in excitotoxicity (see Chapter 10). Hence, before glutamate receptors can be targeted for pharmacologic intervention in schizophrenia, more research must be conducted into the detailed mechanisms by which glutamate may be involved in the pathophysiology of schizophrenia.

## PHARMACOLOGIC CLASSES AND AGENTS

### Mechanism of Action of Antipsychotic Drugs

Fortunately, although the biological basis of schizophrenia remains controversial, a number of drugs are effective in treating the illness. When successful, these medications can lead to a remission of psychosis and allow the patient to integrate into society. Patients only rarely return completely to their premorbid state, however. Drugs used in the management of psychosis are often called **neuroleptics** or **antipsychotics.** Although these terms are frequently used interchangeably, they have slight, yet important, differences in connotation. The term "neuroleptic" emphasizes the drugs' neurological actions that are commonly manifested as side effects of treatment. These complications, often called **extrapyramidal effects,** result from DA receptor blockade in the basal ganglia and, possibly, other areas of the brain. The term "antipsychotic" denotes the ability of these drugs to abrogate psychosis and alleviate disordered thinking in schizophrenic patients. "Antipsychotic" is a more optimistic (albeit somewhat naïve) term because no drugs currently in clinical use have antipsychotic effects without neuroleptic effects.

Until recently, all antipsychotics in clinical use had significant activity as antagonists at dopamine receptors, in particular, at the D2 receptor. The history of these drugs, which are now known as the **typical antipsychotics,** dates back to the approval of **chlorpromazine** in 1954. In the 1960s, as the role of DA in the brain became better understood, the ability of these drugs to block dopaminergic neurotransmission in the CNS was first elucidated. Affinity binding studies performed in the 1980s demonstrated that both therapeutic efficacy and extrapyramidal side effects of the typical antipsychotics correlate directly with the affinity of these drugs for D2 receptors. As shown in Figure 11-10, compounds with higher affinity for D2 receptors, as represented by lower dissociation constants, tend to require smaller doses to control psychotic symptoms and alleviate schizophrenia.

Although the typical antipsychotics block D2 receptors in all of the CNS dopaminergic pathways, their mechanism of action as antipsychotics seems to involve antagonism of mesolimbic, and possibly mesocortical, D2 receptors. As

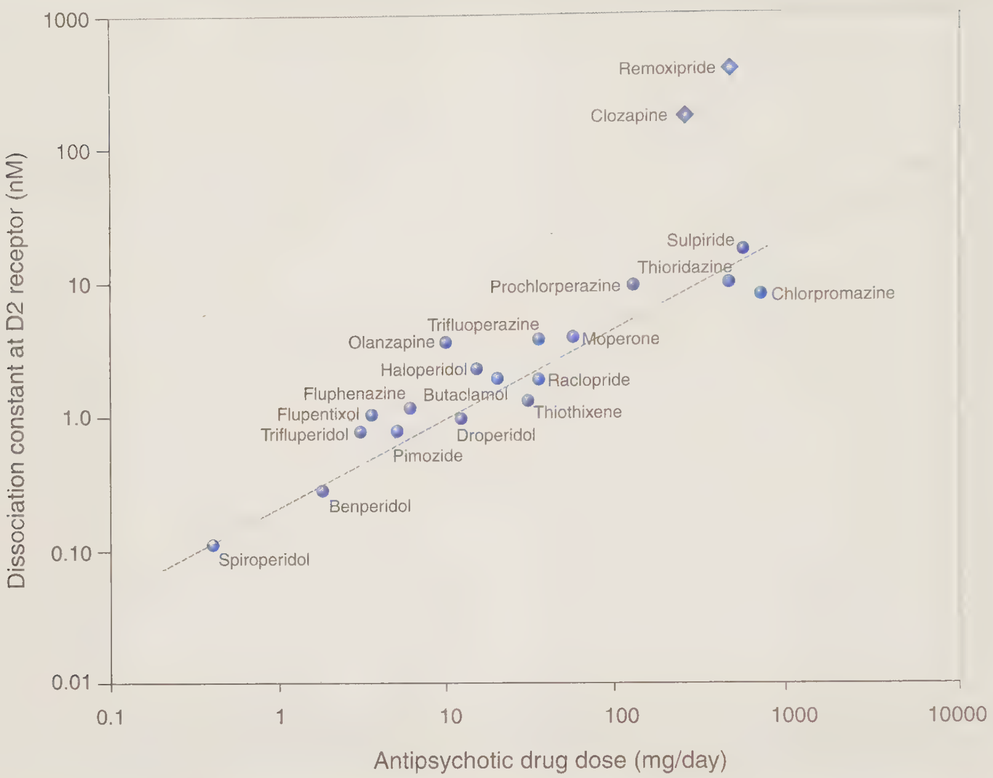


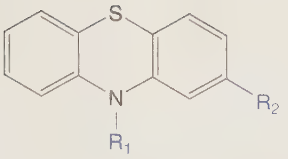
**Figure 11-10. Antipsychotic Potency of Dopamine Receptor Antagonists.** Over at least three orders of magnitude, the clinically effective dose of typical antipsychotics is proportional to the dissociation constant of the drugs at D2 receptors. (Note that a higher dissociation constant represents a lower binding affinity.) Such atypical antipsychotics as clozapine and remoxipride (blue diamonds) are exceptions to this rule; these agents have clinical effects at a dose lower than that predicted by their dissociation constants. Datapoints represent the mean dissociation constant (averaged over multiple studies) at the most typical clinically effective dose. The dotted line represents the best fit to the data for all of the typical antipsychotics (blue circles).

described earlier, one theory holds that the positive symptoms of schizophrenia correlate with hyperactivity of the mesolimbic system; antagonism of mesolimbic dopamine receptors could alleviate these symptoms. The typical antipsychotics are relatively less effective at controlling the negative symptoms of schizophrenia. This relative lack of efficacy at treating the negative symptoms of schizophrenia could relate to the hypothesis that the negative symptoms correlate with hypoactivity of mesocortical neurons because the antagonist action of the antipsychotics would not be expected to correct dopaminergic hypoactivity. Many of the side effects of the typical antipsychotics are likely mediated by binding of these drugs to D2 receptors in the basal ganglia (nigrostriatal pathway) and pituitary gland.

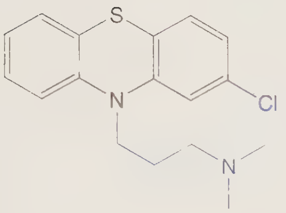
The typical antipsychotics fall into several structural classes, of which the most prominent are the **phenothiazines** and the **butyrophenones** (Fig. 11-11). Chlorpromazine is the prototypical phenothiazine, and **haloperidol** is the most widely used butyrophenone. Despite differences in structure and D2 receptor affinity, all typical antipsychotics have similar clinical efficacies at their standard doses. In general, aliphatic phenothiazines are less potent antagonists at D2 receptors than are phenothiazines functionalized with a piperazine derivative (such as **fluphenazine**), **thioxanthines** (phenothiazines in which a nitrogen in the phenothiazine nucleus is substituted by a carbon), and butyrophenones. For such drugs, a larger dose can overcome a lower in vitro binding affinity for the D2 receptor, so that effectiveness is unaffected by potency at clinically useful doses. However, the potency of the typical antipsychotics is critical in determining the drugs' side effects profile.

## Side Effects

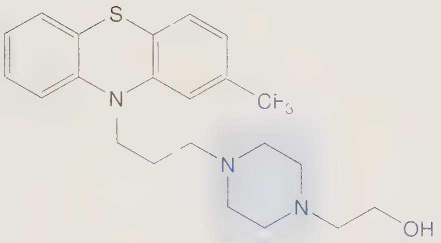
The side effects of typical antipsychotic drugs can be divided into two broad categories: those caused by antagonist action at dopamine D2 receptors outside the mesolimbic and mesocortical systems (the most prominent of these effects are often referred to as "**extrapyramidal effects**"), and those caused by nonspecific antagonist action at other receptor types. Given the broad distribution of dopamine receptors, it is not surprising that dopamine receptor antagonists have a wide range of side effects. As discussed earlier ("Physiology of Nigrostriatal Pathways"), dopaminergic projections from the substantia nigra to the striatum control the initiation of purposeful movement. Because endogenous stimulation of dopamine D2 receptors inhibits the indirect pathway, antagonism of D2 receptors by antipsychotic drugs can disinhibit the indirect pathway and thereby induce Parkinsonian symptoms. Such symptoms are often treated with antiparkinsonian drugs—in particular, amantadine and anticholinergic drugs—because treatment with dopamine agonists could cause a relapse of



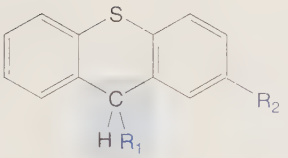
Phenothiazine skeleton



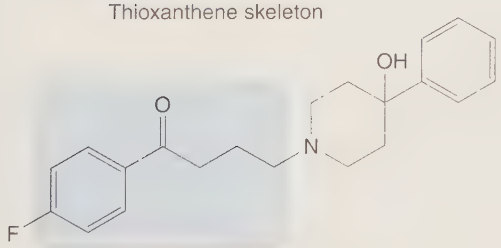
Chlorpromazine



Fluphenazine



Thioxanthene skeleton



Haloperidol (a butyrophenone)

**Figure 11-11. Chemical Structures of the Antipsychotics.** The structure of the phenothiazines is based on a common skeleton, with two variable functional groups. Chlorpromazine, the first approved antipsychotic, has substituted aminopropyl ($R_1$) and chloride ($R_2$) side groups. Piperizine (in blue box)-substituted phenothiazines, such as fluphenazine, are significantly more potent than are aliphatic-substituted phenothiazines, such as chlorpromazine. The fourth structure represents the skeleton of a thioxanthene, which substitutes a carbon (in blue box) for the phenothiazine nitrogen. As illustrated by the structure of haloperidol, butyrophenones (in blue box) are structurally distinct from phenothiazines and thioxanthenes.

schizophrenia symptoms. Severe side effects of antipsychotics include the so-called **neuroleptic malignant syndrome,** characterized by catatonia, stupor, fever, and autonomic instability; myoglobinemia and death occur in about 10% of these cases. Hyperthermia is exacerbated by the actions of the antipsychotics on the hypothalamus, where they interfere with the body's ability to control temperature.

After some time, as striatal D2 receptors become supersensitized, most patients taking antipsychotic drugs experience an amelioration of Parkinsonian side effects. However, after months to years of chronic antipsychotic use, a condition known as **tardive dyskinesia** develops in about 20% of patients. The syndrome is characterized by repetitive, involuntary, stereotyped movements of the facial musculature, arms, and trunk. The exact mechanism is unknown, but it is believed that adaptations resulting in excessive dopaminergic activity are involved. The condition mimics Huntington's chorea, a disease characterized by destruction of the basal ganglia and consequent involuntary choreiform movements. Tardive dyskinesia can be exacerbated by the administration of antiparkinsonian drugs. While no cure exists for tardive dyskinesia, discontinuation of antiparkinsonian drugs can ameliorate the symptoms. In addition, administration of high doses of high-potency antipsychotics can temporarily suppress the disorder, overcoming the adaptive response in the striatal neurons. In many cases, cessation of all antipsychotic medications can reverse the adaptive hypersensitivity of D2 receptors in the striatum and improve the symptoms of tardive dyskinesia.

Some side effects of typical antipsychotics are thought to be caused by antagonist action at dopamine receptors in the pituitary gland, where dopamine tonically inhibits **prolactin** secretion. Antagonism of D2 receptors increases prolactin secretion, which leads to amenorrhea, galactorrhea, and false-positive pregnancy tests in women, and to gynecomastia and decreased libido in men.

Other side effects of antipsychotics result from nonspecific antagonism of muscarinic and α-adrenergic receptors. Antagonism of peripheral muscarinic pathways results in typical anticholinergic effects—dry mouth, constipation, difficulty urinating, and loss of accommodation (see Chapter 7, Cholinergic Pharmacology). α-Adrenergic antagonism can cause orthostatic hypotension and, in men, failure to ejaculate. Sedation can also occur because of inhibition of central α-adrenergic pathways in the reticular activating system. When sedation interferes with normal functioning during chronic antipsychotic use, it is considered an adverse effect. In the acutely psychotic patient, however, sedation may be part of the drug's intended spectrum of action.

The side effect profiles of the typical antipsychotics depend on their potency. High-potency drugs (whose clinical doses are only a few milligrams) tend to have fewer sedative side effects and cause less postural hypotension than drugs with lower potency (i.e., drugs that require high doses to achieve a therapeutic effect). On the other hand, lower-potency antipsychotics tend to cause fewer extrapyramidal side effects. These observations can be rationalized by the fact that high-potency drugs have very high affinity for D2 receptors and, therefore, a higher selectivity of action. Thus, these drugs are more likely to cause side effects mediated by dopamine D2 receptors (i.e., extrapyramidal side effects), and fewer side effects mediated by muscarinic and α-adrenergic receptors (i.e., anticholinergic side effects, sedation, and postural hypotension). Conversely, low-potency antipsychotics do not bind D2 receptors as strongly and cause fewer extrapyramidal side effects, while their lower selectivity results in more prominent anticholinergic and antiadrenergic side effects.

## Pharmacokinetics, Metabolism, and Drug Interactions

As with many drugs active in the CNS, antipsychotics are highly lipophilic. In part because of this lipophilicity, antipsychotics tend to be metabolized in the liver, to have high first-pass metabolism, and to be highly bound to plasma proteins. The drugs are generally formulated as oral or intramuscular dosage forms. The latter are useful in treating acutely psychotic patients who may be a danger to themselves or others, while the oral formulations are generally used for chronic therapy. Elimination half-lives of the antipsychotics are erratic because their kinetics of elimination typically follow a multiphasic pattern and are not strictly first order. In general, however, the half-lives of most typical antipsychotics are on the order of one day, and it is common practice to follow a once-daily dosing regimen.

Two drugs, **haloperidol** and **fluphenazine,** are available as the decanoate esters. These highly lipophilic drugs are injected intramuscularly, where they are slowly hydrolyzed and released. The decanoate ester dosage forms provide a long-acting formulation that can be administered every 3 to 4 weeks. These formulations are particularly useful for treating poorly compliant patients.

Because typical antipsychotics are antagonists at dopamine receptors, it is logical that these drugs should interact prominently with antiparkinsonian drugs that act by increasing synaptic dopamine concentrations (levodopa) or by stimulating dopamine receptors directly (bromocriptine). Specifically, antipsychotics inhibit the action of both of the latter drug classes. In addition, antipsychotics potentiate the sedative effects of benzodiazepines and centrally active antihistamines. Because the latter are pharmacodynamic effects that result from the nonspecific binding of antipsychotics to cholinergic and adrenergic receptors, the low-potency antipsychotics tend to manifest more pronounced sedative effects than their high-potency counterparts. The antipsychotics exhibit relatively few pharmacokinetic drug interactions.

## Atypical Antipsychotics

The so-called atypical antipsychotics have efficacy and side effect profiles that differ from those of the typical antipsychotics. The five principal atypical antipsychotics are **clozapine, olanzapine, quetapine, ziprasidone,** and **risperidone.** All of these drugs are more effective than typical antipsychotics at treating the "negative" symptoms of schizophrenia. In addition, direct comparisons of risperidone and haloperidol have shown that risperidone is more effective than haloperidol at combating the positive symptoms of schizophrenia and preventing a relapse of the active phase of the disease. Atypical antipsychotics cause significantly milder extrapyramidal symptoms than typical antipsychotics; this side effect generally appears only when the drugs are administered in high doses.

The atypical antipsychotics have a relatively low affinity for D2 receptors; unlike the typical antipsychotics, their affinity for D2 receptors does not correlate with their clinically effective dose (Fig. 11-10). Three main hypotheses have emerged to explain this discrepancy. The 5-$HT_2$ hypothesis states that antagonist action at the serotonin 5-$HT_2$ receptor (see Chapter 12), or antagonist action at both 5-$HT_2$ and D2 receptors, is critical for the antipsychotic effect of the atypical antipsychotics. This hypothesis is based on the finding that the FDA-approved atypical antipsychotics are all high-affinity 5-$HT_2$ receptor antagonists. It is not clear, however, how 5-$HT_2$ antagonism contributes to the antipsychotic effect. Moreover, **amisulpride,** an atypical antipsychotic not currently approved for use in the United States, is not a 5-$HT_2$ receptor antagonist. In addition, although some typical antipsychotics also act as antagonists at 5-$HT_2$ receptors, it appears that their clinical effectiveness can be explained based on their affinity for D2 receptors.

The second theory, the D4 hypothesis, is based on the finding that many of the atypical antipsychotics are also dopamine D4 receptor antagonists. This theory suggests that selective D4 antagonism, or a combination of D2 and D4 antagonism, is critical to the mechanism of action of the atypical antipsychotics. D4 receptors are localized to the frontal cortex, medulla, and midbrain (Figs. 11-4 and 11-5), and seem not to be involved in the regulation of movement. This observation is consistent with the clinical experience that atypical antipsychotics exhibit relatively few extrapyramidal side effects. Quetapine does not act as a D4 receptor antagonist, however, so the D4 hypothesis cannot account for the mechanism of action of all atypical antipsychotics.

The final hypothesis states that the atypical antipsychotics exhibit an improved side effect profile because of their relatively fast dissociation from the D2 receptor. As described in Chapter 2, Pharmacodynamics, the binding affinity ($K_d$) of a drug is equal to the ratio of the rate of dissociation ($k_{off}$) to the rate of association ($k_{on}$), as shown in Equation 11-1.

$$D + R \xrightarrow{k_{on}} DR \xrightarrow{k_{off}} D + R$$

$$K_d = \frac{k_{off}}{k_{on}} \qquad \text{Equation 11-1}$$

Because of their rapid off rates, the atypical antipsychotics bind dopamine D2 receptors more transiently than do typical antipsychotics. This could allow the atypical antipsychotics to inhibit the low-level, tonic dopamine release that may occur in the mesolimbic system. However, the drugs would be displaced by a surge of dopamine, as would occur in the striatum during the initiation of movement. Thus, extrapyramidal side effects would be minimized. Because of their relatively high off rates, the drugs tend to have a higher $K_d$ and a lower potency. As with typical antipsychotics that possess a low potency, this results in relatively low selectivity. According to this **"fast dissociation"** hypothesis of atypical antipsychotic action, the 5-$HT_2$ and D4 antagonism are side effects of the lower potency of the atypical antipsychotics and have no bearing on their mechanism of antipsychotic action. Although this theory is attractive in some aspects, it fails to explain the relatively low incidence of prolactin-mediated side effects of the atypical antipsychotics. Recall that prolactin is tonically inhibited by dopamine release in the pituitary gland. One would expect that, because prolactin inhibition is tonic, the atypical antipsychotics would interfere with this process and cause symptoms, such as gynecomastia and galactorrhea.

The atypical antipsychotics are diverse structurally. Their receptor-binding profiles also differ, as summarized in the Drug Summary Table. However, these agents all show combined antagonist properties at dopamine D2 and serotonin 5-$HT_2$ receptors, and most of the drugs are also dopamine D4 receptor antagonists.

Risperidone has combined antagonist properties at dopamine D2 and serotonin 5-$HT_2$ receptors, although it is a stronger serotonergic antagonist. The drug also antagonizes $\alpha_1$-adrenergic, $\alpha_2$-adrenergic, and histamine $H_1$ receptors with relatively high affinity. Its side effect profile is predictable from its broad pharmacologic profile.

Clozapine binds dopamine D1–D5 receptors as well as serotonin 5-$HT_2$ receptors; in addition, it blocks $\alpha_1$-adrenergic, histamine $H_1$, and muscarinic receptors. Clozapine has been used therapeutically in patients who have failed other antipsychotic drugs, whether for lack of efficacy or intolerable side effects. Clozapine has not been used as a first-line agent because of a small but significant risk of agranulocytosis (approximately 0.8% per year). Thus, the administration of clozapine requires frequent monitoring of white blood cell counts.

Olanzapine, ziprasidone, and quetapine are also combined D2 and 5-$HT_2$ receptor antagonists, and each binds a number of other receptors. Their pharmacologic profiles are summarized in the Drug Summary Table.

## *Conclusion and Future Directions*

Treatments for both Parkinson's disease and schizophrenia modulate dopaminergic neurotransmission in the CNS. Parkinson's disease, which results from the degeneration of dopaminergic neurons that project to the striatum, causes resting tremor and bradykinesia. In this disease, the direct pathway, which helps enable movement, is understimulated; whereas, the indirect pathway, which inhibits movement, becomes disinhibited. Pharmacologic treatment of Parkinson's disease depends on agents that increase dopamine release or activate dopamine receptors in the caudate and putamen, and thereby, help restore the balance between the direct and indirect pathways.

In comparison, schizophrenia is treated by inhibiting dopamine receptors at various sites in the limbic system. Unfortunately, the pathophysiology of schizophrenia is not fully understood; this lack of knowledge about etiology limits rational drug development. The clinical effectiveness of the various antipsychotic agents has provided useful clues, however, and has formed the basis of the dopamine theory of schizophrenia, which argues that dysregulated levels of dopamine in the brain play a role in the pathophysiology of the disease. The effectiveness of newer, atypical antipsychotic agents, which affect the function of several different receptor types, has highlighted the fact that the dopamine theory is a simplification. The atypical agents represent an attractive new modality for treating schizophrenia because they have fewer extrapyramidal side effects and are more effective than the typical antipsychotics.

Future developments in the treatment of Parkinson's disease and schizophrenia are focused on creating more selective agents within the current drug classes and on better elucidating the underlying pathophysiology of the disorders. New dopamine receptor agonists with higher selectivity, particularly those that bind D1 receptors, may one day provide more effective treatment for Parkinson's disease with milder side effects. The development of newer antipsychotics with increased receptor selectivity may similarly expand the therapeutic options for treating schizophrenia. Because Parkinson's disease results from the death of dopaminergic neurons, much effort is currently directed at neuroprotective drugs that may slow the progression of the disease. One such effort is focused on the use of such trophic factors as glial-cell–derived neurotrophic factor (GDNF), which has been shown to increase the survival of dopaminergic neurons in vitro and to improve Parkinsonian symptoms in monkeys. Further research into a potential role for a glutamate deficit in the pathophysiology of schizophrenia may yield new therapeutics for this disorder. For example, the development of selective glutamate receptor agonists may one day complement or even replace the use of dopamine receptor antagonists. Another important advance in the treatment of schizophrenia will likely result from the elucidation of models for the mechanism of the atypical antipsychotics, which will allow rational development of more effective atypicals.

## *Suggested Readings*

Ahlskog EJ. Parkinson's disease: Medical and surgical treatment. Neurol Clin 2001;19:579. (*A concise yet comprehensive review of the current medical and surgical treatment of Parkinson's disease. It also touches on future directions for research.*)

Diagnostic and Statistical Manual of Mental Disorders. 4th Ed. Washington, DC: American Psychiatric Association, 1994. (*A description of the criteria needed for a diagnosis of schizophrenia (as well as other mental disorders).*)

Feldman RS, Meyer JS, Quenzer LF. Principles of Neuropsychopharmacology. Sunderland, MA: Sinauer Associates, Inc. 1997. (*An in-depth treatment of catecholamine neurotransmission, Parkinson's disease, schizophrenia, and dopamine system therapies. It is well written, clear, and easy to follow.*)

Freedman R. Drug therapy: Schizophrenia. N Engl J Med 2003; 349:1738–1749. (*Discusses the clinical use of many of the drugs used to treat schizophrenia, including the atypical agents.*)

Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiat 2001; 158:1367–1377. (*Discusses the evidence for the hypothesis that glutamate plays a critical role in the pathophysiology of schizophrenia.*)

Jenner P. Pharmacology of dopamine agonists in the treatment of Parkinson's disease. Neurology 2002;58(Suppl 1):S1–8. (*This issue of Neurology contains eleven review articles discussing the use of dopaminergic agonists and other agents in the treatment of Parkinson's disease.*)

Kandel ER, Schwartz JR, Jessell TM, eds. Principles of Neural Science. New York: McGraw-Hill, 2000. (*A classic book in neuroscience, this volume has an excellent chapter on the basal ganglia and the pathophysiology of Parkinson's disease.*)

Kapur S, Seeman P. Does fast dissociation from the dopamine D2 receptor explain the action of atypical antipsychotics?: A new hypothesis. Am J Psychiat 2001;158:360–369. (*Discusses the evidence for the "fast dissociation" hypothesis for atypical antipsychotic action.*)

Thaker GK, Carpenter WT. Advances in schizophrenia. Nature Med 2001;7:667–671. (*Discusses some of the recent research into the pathophysiology and pharmacology of schizophrenia.*)

## Drug Summary Table Pharmacology of Dopaminergic Neurotransmission

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **DOPAMINE PRECURSORS**<br>*Mechanism—Provide substrate for increased dopamine synthesis, while bypassing rate-limiting step in dopamine synthesis* | | | | |
| **Levodopa** | Treatment of Parkinson's disease (PD) | Choreiform or dystonic movements | MAO inhibitors, patients with suspected malignant melanoma, patients with history of schizophrenia | Low availability to CNS due to peripheral metabolism to DA when administered alone. Usually administered in combination with carbidopa |
| **DOPAMINE AGONISTS**<br>*Mechanism—Bind to and activate dopamine receptors* | | | | |
| **Bromocriptine** | Treatment of PD, hyperprolactinemia, acromegaly | Choreiform or dystonic movements, drowsiness | Uncontrolled hypertension, sensitivity to ergot alkaloids, pregnancy | May be associated with increased incidence of hypertension, seizure, stroke, and MI in postpartum women |
| **Pramipexole** | Treatment of PD | Somnolence | Avoid other sedating medications | More potent at D3 than D2 dopamine receptors |
| **Ropinirole** | Treatment of PD | | | More potent at D3 than D2 dopamine receptors |
| **INHIBITORS OF LEVODOPA OR DOPAMINE METABOLISM**<br>*Mechanism—Inhibit peripheral breakdown of levodopa or inhibit the breakdown of dopamine in the CNS* | | | | |
| **Carbidopa** | Treatment of PD; administered with levodopa | Can exacerbate levodopa side effects | | Dopa decarboxylase inhibitor; must be administered as a combined medication with levodopa to have antiparkinsonian effect; increases CNS availability of levodopa |
| **Selegiline** | Second line treatment of PD | Symptoms of DA excess | Tricyclic antidepressants and selective serotonin reuptake inhibitors | Selectively inhibits breakdown of DA by MAO-B, which predominates in striatum |
| **Tolcapone** | Treatment of PD | Liver failure, dyskinesia | Nonselective MAO inhibitors | Inhibits breakdown of levodopa by COMT in CNS due to high blood–brain barrier permeation |
| **Entacapone** | Adjunct in the treatment of PD | Rhabdomyolysis, dyskinesia, somnolence | MAO inhibitors | Inhibits breakdown of levodopa by COMT in periphery, increasing CNS availability |
| **MISCELLANEOUS ANTIPARKINSONIAN MEDICATIONS**<br>*Mechanism—Various, see Notes* | | | | |
| **Amantadine** | PD, Influenza A | Insomnia, anxiety | May exacerbate mental illness in patients with psychiatric illness or substance abuse problems | Used to treat dyskinesias; mechanism not understood |
| **Trihexyphenidyl** | PD, Extrapyramidal syndrome | Antimuscarinic side effects | Atherosclerotic vascular disease | Muscarinic receptor antagonist; reduces cholinergic tone in CNS; primarily affects tremor component of PD |

|  |  |  |  |  |
|---|---|---|---|---|
| **PHENOTHIAZINES (AND DERIVATIVES)**<br>*Mechanism—Dopamine D2 receptor antagonists (low and medium potency)* |  |  |  |  |
| **Chlorpromazine** | Psychosis; mania; nausea and vomiting | All phenothiazines and derivatives can cause:<br>Extrapyramidal effects<br>Effects of increased prolactin<br>Antimuscarinic effects<br>Orthostatic hypotension |  | Chlorpromazine is the prototype of the typical antipsychotics |
| **Thioridazine** | Schizophrenia (second line agent) |  | Patients with long QT interval | May prolong QT interval |
| **Mesoridazine** | Schizophrenia (second line agent) |  | Patients with long QT interval | May prolong QT interval |
| **Perphenazine** | Psychosis; nausea and vomiting |  | Bone marrow suppression; CNS depression |  |
| **Fluphenazine** | Psychotic disorders; schizophrenia |  |  | Available in a depot formulation |
| **Thiothixene** | Psychotic disorders |  | Bone marrow suppression. CNS depression |  |
| **Trifluoperazine** | Psychotic disorders: generalized anxiety disorder |  |  |  |
| **Chlorprothixene** | Psychotic disorders |  | Ethanol |  |
| **BUTYROPHENONES**<br>*Mechanism—Dopamine D2 receptor antagonists (high potency)* |  |  |  |  |
| **Haloperidol** | Treatment of psychoses; control of tics and vocal utterances of Tourette's disorder | All butyrophenones can cause:<br>Extrapyramidal effects<br>Effects of increased prolactin<br>Antimuscarinic effects |  | May be used for the emergency sedation of severely agitated or delirious patients |
| **Haloperidol decanoate** | Treatment of schizophrenia |  |  | Depot formulation allows for dosing every 3 weeks |
| **Droperidol** | Nausea and vomiting; anesthesia adjunct |  |  | Used in conjunction with analgesics (fentanyl) to produce neuroleptalgesia (see Chapter 15)<br>Causes retinal pigmentation |
| **OTHER TYPICAL ANTIPSYCHOTICS**<br>*Mechanism—Dopamine D2 receptor antagonists* |  |  |  |  |
| **Loxapine** | Psychotic disorders | Loxapine, molindone and pimozide can cause:<br>Extrapyramidal effects<br>Effects of increased prolactin<br>Antimuscarinic effects<br>Orthostatic hypotension | CNS depression; coma | IM administration more likely to cause hypotension |
| **Molindone** | Psychotic disorders |  | Inhibitors of CYP3A4 |  |
| **Pimozide** | Psychotic disorders, Tourette's disorder |  | Patients with long QT interval |  |

*(Continued)*

**Drug Summary Table** **Pharmacology of Dopaminergic Neurotransmission** *(Continued)*

| Drug | Clinical Uses | Side Effects | Drug Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ATYPICAL ANTIPSYCHOTICS** | | | | |
| *Mechanism—Dopamine D2 receptor antagonists, serotonin 5-HT$_2$ receptor antagonists; other CNS receptor antagonists* | | | | |
| **Risperidone** | Psychosis | QT prolongation, weight gain | Patients with long QT interval | Binds to D2, 5-HT$_2$, $\alpha_1$, $\alpha_2$, H1 receptors |
| **Clozapine** | Schizophrenia refractory to other antipsychotics | Weight gain, agranulocytosis | Leukopenia | Binds to D1–D5, 5-HT$_2$, $\alpha_1$, H1, muscarinic receptors |
| **Olanzapine** | Psychosis, mania of bipolar disorder | Weight gain | | Binds to D1–D4, 5-HT$_2$, H1, $\alpha_1$, M$_1$–M$_5$ receptors |
| **Quetapine** | Psychosis | | | Binds to D1, D2, 5-HT$_1$, 5-HT$_2$, $\alpha_1$, $\alpha_2$, H1 receptors |
| **Ziprasidone** | Schizophrenia | | | Binds to D2, 5-HT$_1$, 5-HT$_2$, $\alpha_1$, H1 receptors |
| **Aripiprazole** | Schizophrenia | Seizures, change in temperature regulation | Inhibitors of CYP3A4 | D2 and 5-HT$_{1A}$ partial agonist; 5-HT$_{2A}$ antagonist |

# 12

# Pharmacology of Affective Disorders

Jay H. Chyung, Roy Perlis, and Scott L. Rauch

Introduction
Case
Biochemistry and Physiology of Central Adrenergic and Serotonergic Neurotransmission
  Principles of Central Amine Neurotransmission
  Neurotransmitter Synthesis
    *Serotonin*
    *Norepinephrine*
  Regulation of Neurotransmitter Levels
  Receptors
    *Serotonin*
    *Norepinephrine*
  Physiologic Actions
Pathophysiology of Affective Disorders
  Diagnostic Criteria for Affective Disorders
  The Monoamine Theory of Depression
    *Limitations of the Monoamine Theory*
    *Role of Monoamine Receptor Sensitivity Changes in the Therapeutic Action of Antidepressants*
  Bipolar Disorder
Pharmacologic Classes and Agents
  Tricyclic Antidepressants (TCAs)
    *Mechanism of Action*
    *Side Effects*
    *Pharmacokinetics*
  Selective Serotonin Reuptake Inhibitors (SSRIs)
    *Mechanism of Action*
    *Side Effects*
    *Pharmacokinetics*
  MAO Inhibitors (MAOIs)
    *Mechanism of Action*
    *Side Effects*
    *Pharmacokinetics*
  Other Antidepressants
  Lithium
    *Mechanisms of Action*
    *Side Effects*
    *Pharmacokinetics*
Conclusion and Future Directions
Suggested Readings

## INTRODUCTION

This chapter discusses the major mood disorders and the drugs used to treat them. The chapter begins with a discussion of central noradrenergic and serotonergic neurotransmission. Chapter 8, Adrenergic Pharmacology, discussed norepinephrine as a neurotransmitter in the sympathetic nervous system. This chapter extends the concepts presented there to the central nervous system. In addition, this chapter introduces the neurotransmitter **serotonin** (5-hydroxytryptamine), which is a target for many of the drugs used to treat depression. The chapter then turns to the pathophysiology of major depressive disorder (MDD) and the drugs used to treat MDD, which affect the neurotransmission of central noradrenergic and serotonergic neurons. Bipolar disorder (BPD) and **lithium,** a drug commonly used to treat BPD, are also discussed briefly.

The major mood disorders are defined by the presence of depressive and/or manic episodes. Patients who have experienced at least one manic episode, with or without an additional history of depressive episodes, are said to have BPD; patients with major depressive episodes and no history of mania are said to have MDD. The lifetime prevalence of MDD is approximately 17%; whereas, that of BPD is 1%–2%. There is a particularly strong heritable risk in BPD. MDD can occur as an isolated illness or in association with other diseases such as stroke, dementia, diabetes, cancer, and coronary artery disease. In addition, many classes of drugs can exacerbate depression. Both MDD and BPD are major causes of morbidity worldwide, resulting in lost productivity and substantially increased use of medical resources. Affective illness is associated with an increased risk of suicide; rates of completed suicide are estimated to be as high as 15% among untreated patients. In the majority of suicides, a physician (not necessarily a psychiatrist) will have seen the patient less than 1 month before the suicide.

## Case

Mary R. is a 27-year-old office worker who presents to her primary care physician, Dr. Lee, with an 18-lb. weight loss over the previous 2 months. Mary tearfully explains that she is plagued by near-constant feelings of sadness and by a sense of helplessness and inadequacy at work. She feels so terrible that she has not had a good night of sleep in more than a month. She no longer enjoys living and has recently become scared when new thoughts of suicide enter her mind. Mary tells Dr. Lee that she had felt like this once before, but that it had passed. Dr. Lee asks Mary about her sleep patterns, appetite levels, ability to concentrate, energy level, mood, interest level, and feelings of guilt. He asks her specific questions about thoughts of suicide, particularly whether she has formed a specific plan and whether she has ever attempted suicide. Dr. Lee explains to Mary that she has major depressive disorder, likely caused by a chemical imbalance in her brain, and he prescribes the antidepressant **fluoxetine.**

Two weeks later, Mary calls to complain that the medicine is not working. Dr. Lee encourages Mary to continue taking the medicine, and after 2 more weeks, Mary begins to feel better. She no longer feels sad and demoralized; the feelings of helplessness and inadequacy that previously plagued her have melted away. In fact, when she returns to see Dr. Lee 6 weeks later, she reports feeling better than ever. She no longer needs much sleep, and is always full of energy. In fact, she is now convinced that she is the most intelligent person in her company. Mary proudly tells Dr. Lee that she has recently purchased a new sports car and gone on a large shopping spree. Dr. Lee tells Mary that she may be having a manic episode and, in consultation with a psychiatrist, prescribes **lithium** and tapers the fluoxetine. Mary is hesitant to take the new medication, arguing that she feels fine and that she is concerned about the side effects of lithium.

### QUESTIONS

1. How is a depressive episode different from occasionally "feeling blue"?
2. How do fluoxetine and other antidepressants work?
3. Why is there a delay in the onset of fluoxetine's therapeutic effect?
4. What caused Mary's mania? Why is it necessary to treat BPD if the patient feels good?
5. What specific concerns might Mary have about the side effects of lithium?

## BIOCHEMISTRY AND PHYSIOLOGY OF CENTRAL ADRENERGIC AND SEROTONERGIC NEUROTRANSMISSION

### PRINCIPLES OF CENTRAL AMINE NEUROTRANSMISSION

MDD has been linked to dysfunction of the neurotransmitter pathways involving serotonin (5-hydroxytryptamine, or 5HT) and norepinephrine (NE). The serotonin and norepinephrine neurotransmitter systems fall into the class of central neurotransmitter systems known as **diffusely projecting systems** (see Chapter 6, Principles of Nervous System Physiology and Pharmacology), which modulate the firing of neurons in a global and diffuse manner.

The primary mode of neurotransmitter release from central serotonergic and adrenergic systems is from varicosities. Unlike synapses, which form tight contacts with specific target neurons, varicosities release large amounts of neurotransmitter from vesicles into the extracellular space, establishing concentration gradients of neurotransmitter in the projection areas of the varicosities. 5HT-containing cells within the raphe nuclei and NE-containing cells within the locus ceruleus project broadly throughout the cerebral cortex and autoregulate their own release. This autoregulation results in coordinated firing, which causes spontaneous and synchronous waves of activity that can be measured as firing frequencies. The cells within the **raphe nuclei** usually fire at rates between 0.3 and 7 spikes per second, and the cells within the **locus ceruleus** fire at rates between 0.3 and 4.4 spikes per second. Because the frequency of firing does not change rapidly and the quanta of neurotransmitter released with each discharge are fairly well conserved, the neurotransmitter concentration in the vicinity of the varicosities is maintained within a narrow range.

The mean concentration establishes the baseline **tone** of activity in the target neurons that receive 5HT and NE projections. In addition, specific stimuli can elicit rapid bursts of firing, superimposed on the baseline tonic activity, which provide additional information content. Diffusely projecting systems can, thus, provide two types of information: a rapid and discrete neuronal firing akin to more traditional neurotransmission, and a slower tonic firing frequency that presumably allows for integration of information over a longer period of time.

With the exception of one serotonergic receptor subtype that is a ligand-gated channel, serotonergic and adrenergic receptors signal via G protein-coupled second-messenger systems to alter the excitability of neurons. In general, these G protein-coupled receptors influence the responses of target neurons to other synaptic inputs. G protein signaling can alter neuron responses quickly (on the order of seconds to minutes) by altering the responses of ion channels and other second-messenger system cascades to stimuli. Over the course of hours to days, G protein-coupled signaling systems can also regulate, at the transcriptional level, the quantity and nature of the proteins that participate in signal transduction cascades. Through these mechanisms, the diffusely projecting systems alter the firing threshold of their neuronal targets.

## NEUROTRANSMITTER SYNTHESIS

### Serotonin

Serotonin is synthesized from the amino acid tryptophan by the enzyme **tryptophan hydroxylase** (TPH), which converts tryptophan to **5-hydroxytryptophan. Aromatic L-amino acid decarboxylase** then converts 5-hydroxytryptophan to serotonin (Fig. 12-1A). These enzymes are present throughout the cytoplasm of serotonergic neurons, both in the cell body and in cell processes. Serotonin is also concentrated and stored within vesicles located in axons, cell bodies, and dendrites (see below).

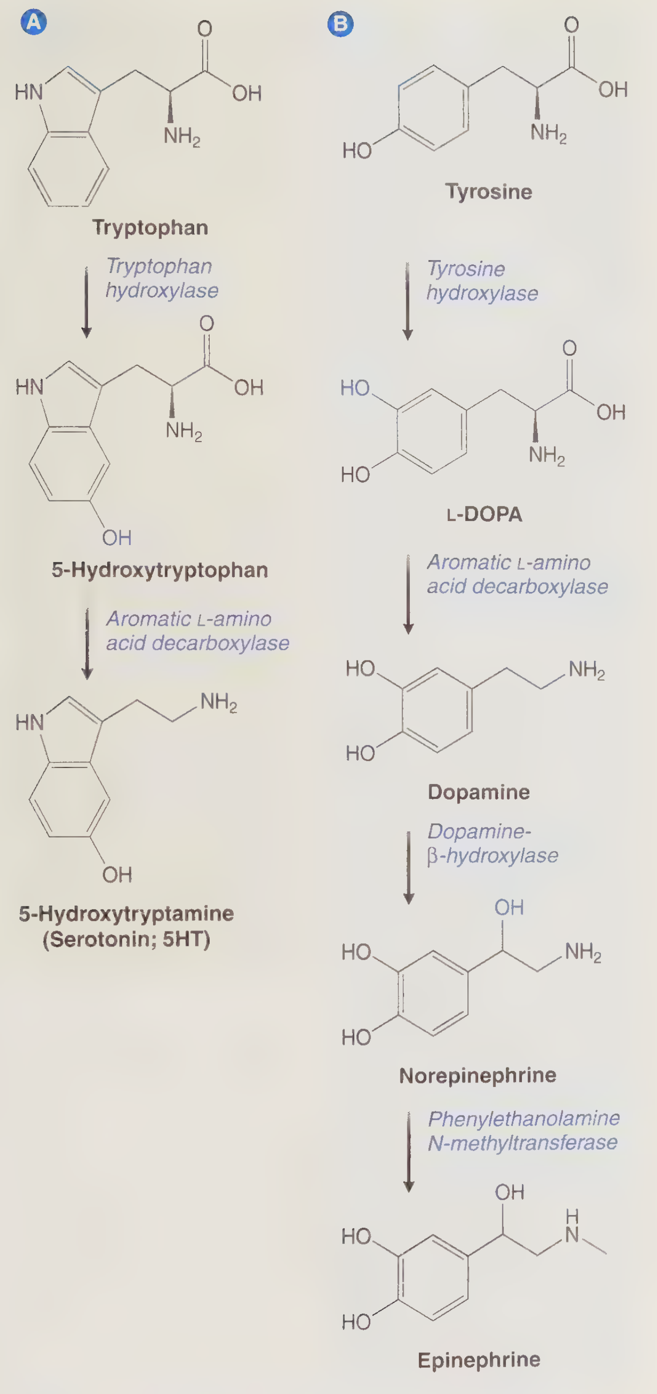

A
Tryptophan
Tryptophan hydroxylase
5-Hydroxytryptophan
Aromatic L-amino acid decarboxylase
5-Hydroxytryptamine (Serotonin; 5HT)
B
Tyrosine
Tyrosine hydroxylase
L-DOPA
Aromatic L-amino acid decarboxylase
Dopamine
Dopamine-β-hydroxylase
Norepinephrine
Phenylethanolamine N-methyltransferase
Epinephrine
HN
OH
NH2
O
HO

**Figure 12-1. Synthesis of Serotonin and Norepinephrine.** **A.** 5-Hydroxytryptamine (serotonin) is synthesized from the amino acid tryptophan in two steps: the hydroxylation of tryptophan to form 5-hydroxytryptophan, and the subsequent decarboxylation of this intermediate to produce 5-hydroxytryptamine (5HT). Tryptophan hydroxylase is the rate-limiting enzyme in this pathway. **B.** Norepinephrine is synthesized from the amino acid tyrosine in a three-step process similar to the synthetic pathway for serotonin. Tyrosine is first oxidized to L-DOPA by the enzyme tyrosine hydroxylase and then decarboxylated to dopamine. After dopamine is transported into the synaptic vesicle, it is hydroxylated by the enzyme dopamine β-hydroxylase to form norepinephrine. Interestingly, the same enzyme decarboxylates 5-hydroxytryptophan and L-DOPA; it is known generically as aromatic L-amino acid decarboxylase. Tyrosine hydroxylase is the rate-limiting enzyme in this pathway.

## Norepinephrine

Norepinephrine is synthesized and stored within vesicles located in axon terminals, cell bodies, and dendrites. The biochemistry of NE synthesis is described in Chapter 8, and is summarized in Figure 12-1B.

## REGULATION OF NEUROTRANSMITTER LEVELS

The metabolic cycles of both NE (Fig. 12-2) and 5HT (Fig. 12-3) involve neurotransmitter synthesis, uptake into synaptic vesicles, exocytosis, reuptake into the cytoplasm, and either reuptake into vesicles or degradation. Importantly, regulation of the levels of 5HT and NE neurotransmission can occur at any of these steps.

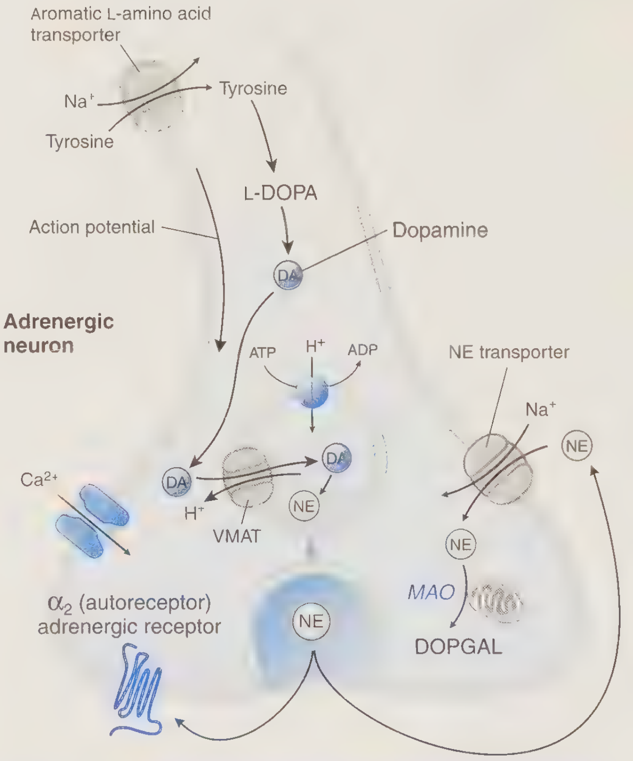

Aromatic L-amino acid transporter
Na⁺
Tyrosine
Tyrosine
L-DOPA
Action potential
Dopamine
DA
Adrenergic neuron
ATP
H⁺
ADP
NE transporter
Na⁺
NE
Ca²⁺
DA
DA
H⁺
NE
VMAT
NE
MAO
α2 (autoreceptor) adrenergic receptor
NE
DOPGAL

**Figure 12-2. Presynaptic Regulation of Norepinephrine Neurotransmission.** Norepinephrine in the synaptic vesicle is derived from two sources. First, dopamine synthesized from tyrosine is transported into the vesicle by the vesicular monoamine transporter (VMAT). Inside the vesicle, dopamine is converted to norepinephrine by dopamine-β-hydroxylase. Second, recycled NE is transported from the cytoplasm into the vesicle, also by VMAT. Neurotransmission is initiated by an action potential in the presynaptic neuron, which eventually causes synaptic vesicles to fuse with the plasma membrane in a $Ca^{2+}$-dependent manner. NE is removed from the synaptic cleft by a selective norepinephrine transporter (NET) as well as by nonselective reuptake transporters (not shown). NE can stimulate $\alpha_2$-adrenergic autoreceptors to provide feedback inhibition. Cytoplasmic NE that is not sequestered in synaptic vesicles by VMAT is instead degraded by monoamine oxidase (MAO) on the outer mitochondrial membrane to 3,4-dihydroxyphenylglycoaldehyde (DOPGAL).

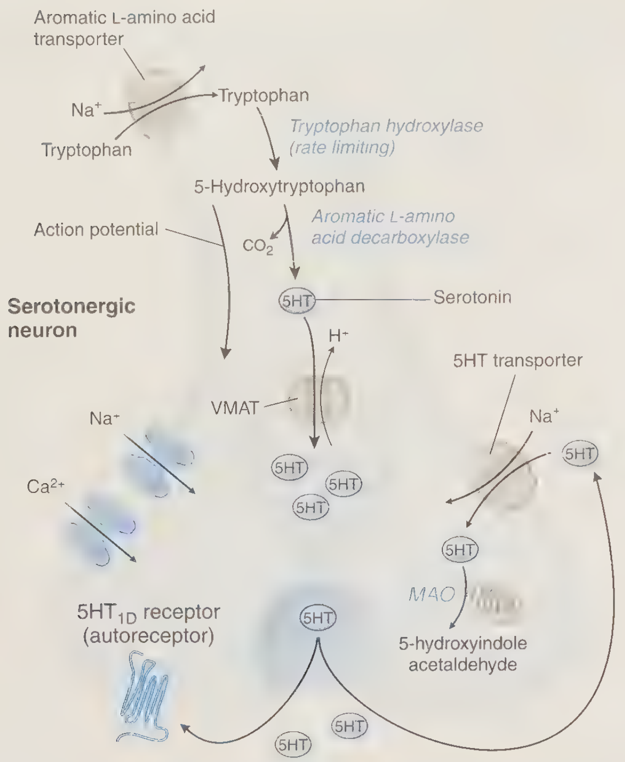

Aromatic L-amino acid transporter
Na$^+$
Tryptophan
Tryptophan
Tryptophan hydroxylase (rate limiting)
5-Hydroxytryptophan
Action potential
CO$_2$
Aromatic L-amino acid decarboxylase
Serotonergic neuron
5HT
Serotonin
H$^+$
5HT transporter
VMAT
Na$^+$
Na$^+$
Ca$^{2+}$
5HT
MAO
5HT$_{1D}$ receptor (autoreceptor)
5-hydroxyindole acetaldehyde

**Figure 12-3. Presynaptic Regulation of Serotonin Neurotransmission.** Serotonin (5HT) is synthesized from tryptophan in a two-reaction pathway: the rate-limiting enzyme is tryptophan hydroxylase. Both newly synthesized and recycled 5HT are transported from the cytoplasm into synaptic vesicles by the vesicular monoamine transporter (VMAT). Neurotransmission is initiated by an action potential in the presynaptic neuron, which eventually causes synaptic vesicles to fuse with the plasma membrane in a Ca$^{2+}$-dependent manner. 5HT is removed from the synaptic cleft by a selective 5HT transporter (5HTT) as well as by nonselective reuptake transporters (not shown). 5HT can stimulate 5HT$_{1D}$ autoreceptors to provide feedback inhibition. Like NE, cytoplasmic 5HT is either sequestered in synaptic vesicles by VMAT or degraded by mitochondrial MAO.

For both monoamines, the first synthetic step is rate-limiting. Thus, NE synthesis is rate-limited by **tyrosine hydroxylase** (TH), and 5HT synthesis by **tryptophan hydroxylase** (TPH). Both enzymes are tightly regulated by inhibitory feedback via autoreceptors. NE and 5HT presynaptic autoreceptors respond to increased NE and 5HT neurotransmission by increasing G protein signaling, which leads to a decrease in the level of cAMP, resulting in a decrease in the activity of protein kinase A and CaM kinase II. Because phosphorylation increases the activity of TH and TPH, the decrease in kinase activity results in decreased NE and 5HT synthesis.

NE and 5HT are transported into vesicles using the same vesicular monoamine transporter (VMAT). The transporter is a nonspecific monoamine transporter that is also important for the vesicular packaging of dopamine (DA) and epinephrine (E). Monoamines are pumped into the vesicles via a transmembrane proton–monoamine antiporter. The proton gradient is maintained by a proton–ATPase pump that also resides in the vesicle membrane. **Reserpine** binds irreversibly to the vesicular monoamine transporter, and therefore, prevents the uptake of DA, NE, E, and 5HT into vesicles. Reserpine treatment also leads to disintegration of the vesicles, releasing monoamines into the cytoplasm for subsequent degradation.

As discussed above, 5HT and NE are released tonically from neuronal varicosities. Through inhibitory presynaptic autoreceptors, an increase in 5HT or NE neurotransmission leads to a reduction in the frequency of firing, to maintain the total quantity of neurotransmitter released over time within a narrow range. In response to rapid firing, inhibitory autoreceptor signaling also leads to a decrease in the quanta of neurotransmitter released per action potential.

Selective reuptake transporters recycle 5HT and NE from the synaptic cleft back into the presynaptic neuron. Both reuptake transporters are twelve-transmembrane-spanning proteins that couple neurotransmitter transport to the transmembrane sodium gradient. Unlike VMAT, which is a nonspecific monoamine transporter, the individual monoamine reuptake transporters show selectivity, high affinity, and low capacity for each monoamine. The selective monoamine transporters, which include the **serotonin transporter** (5HTT), **norepinephrine transporter** (NET), and **dopamine transporter** (DAT), are also capable of transporting the other monoamines, although less efficiently. Other, nonselective reuptake transporters have a lower affinity but a higher capacity for the various neurotransmitters. Amphetamines can both reverse vesicular uptake of monoamines from the cytoplasm by dissipating the proton gradient across the vesicle membrane and stimulate the release of monoamines from the cytoplasm into the extracellular space by reversing the directionality of the selective monoamine transporter in the plasma membrane of the presynaptic neuron.

Once NE and 5HT are returned to the neuronal cytoplasm, the neurotransmitter may undergo either reuptake into vesicles—mediated by VMAT—or degradation by the **monoamine oxidase** (MAO) system. MAOs are mitochondrial enzymes that regulate the levels of monoamines in neural tissues and inactivate circulating and dietary monoamines (such as tyramine) in the liver and gut. MAO-A degrades 5HT, NE and DA, and MAO-B degrades DA more than 5HT or NE. Monoamine oxidases inactivate monoamines by oxidative deamination, using a functional flavin moiety as an electron acceptor. **Catechol-O-methyltransferase** (COMT) in the extracellular space is another important degradation pathway for monoamines, although COMT plays a less significant role in the CNS than it does peripherally.

## RECEPTORS

### Serotonin

Many subtypes of 5HT receptors have been discovered, and all but one are G protein-coupled (Table 12-1). In general, the 5HT$_1$ class of receptors inhibits adenylyl cyclase, the 5HT$_2$ class increases phosphoinositol turnover, and the 5HT$_4$, 5HT$_6$, and 5HT$_7$ classes stimulate adenylyl cyclase. The only known ligand-gated ion channel is the 5HT$_3$ receptor, although several 5HT receptor subtypes have yet to be characterized. The receptors that seem to be most important for clinical depression are the 5HT$_{1A}$, 5HT$_{1D}$, 5HT$_{2A}$, and 5HT$_{2C}$ receptors. 5HT$_{1A}$ is expressed both on serotonergic cell bodies in the raphe nuclei and on postsynaptic neurons in the hippocampus, and its activation results in decreased cAMP levels. Presynaptic 5HT$_{1D}$ mediates the autoinhibitory mech-

**TABLE 12-1** Signaling Mechanisms of Norepinephrine and Serotonin Receptor Subtypes

| NE RECEPTOR SUBTYPE | SIGNALING MECHANISMS |
|---|---|
| $\alpha_1$ | ↑ IP$_3$, DAG |
| $\alpha_2$* | ↓ cAMP |
| $\beta_{1,2}$ | ↑ cAMP |
| **5HT RECEPTOR SUBTYPE** | |
| 5HT$_{1A,B,D,E,F}$* | ↓ cAMP |
| 5HT$_{2A,B,C}$ | ↑ IP$_3$, DAG |
| 5HT$_3$ | Ligand-gated ion channel |
| 5HT$_{4,6,7}$ | ↑ cAMP |

Abbreviations: cAMP = cyclic AMP; IP$_3$ = inositol 1,4,5-trisphosphate; DAG = diacylglycerol

*$\alpha_2$ adrenergic and 5HT$_{1D}$ are presynaptic autoreceptors important for feedback inhibition.

anisms of 5HT neurotransmission at axon terminals. 5HT$_{2A}$ and 5HT$_{2C}$ signaling is excitatory, lowering the threshold for neuronal firing. The various receptor subtypes are expressed differentially throughout the brain. For example, a subset of 5HT projections to the cortex stimulates postsynaptic 5HT$_2$ receptors, while other projections to the limbic system stimulate postsynaptic 5HT$_{1A}$ receptors. There is considerable overlap of receptor subtype expression, however, and the significance of this overlap is not well understood.

### Norepinephrine

Nine subtypes of NE receptors have been characterized. Of these, the most important are $\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_2$ (Table 12-1). Presynaptic expression of the $\alpha_2$ receptor is critical for the autoinhibitory actions of NE neurotransmission. The NE receptors are described in detail in Chapter 8.

## PHYSIOLOGIC ACTIONS

Monoamines play a critical role in modulating mood, the sleep–wake cycle, motivation, pain perception, and neuroendocrine function. Serotonergic projections to the spinal cord are involved in pain perception, visceral regulation, and motor control, while projections to the forebrain are important for modulating mood, cognition, and neuroendocrine function. The noradrenergic system modulates vigilance, stress responses, neuroendocrine function, pain control, and sympathetic nervous system activity. Thus, beyond their roles in mood regulation, the serotonergic and noradrenergic systems are involved in many other complex neuropsychiatric processes.

## PATHOPHYSIOLOGY OF AFFECTIVE DISORDERS

Major depressive disorder and bipolar disorder are both characterized by extremes in mood. Major depressive disorder is typified by recurrent major depressive events, and bipolar disorder is defined by the presence of mania. In addition, a number of other disorders involve combinations or less extreme manifestations of depression and mania. Current molecular theories for the etiology of depression center on the monoamine hypothesis, and theories for the etiology of mania arise mainly from the inferred actions of lithium, a primary agent used to treat mania. However, because the underlying etiologies of these disorders are still not well understood at a molecular level, the criteria for diagnosis rely primarily on clinical evaluation. The American Psychiatric Association diagnostic criteria for major depressive disorder and bipolar disorder are listed in Boxes 12-1 and 12-2.

**BOX 12-1.** Diagnostic and Statistical Manual of Mental Disorders, Text Revision Criteria for Major Depressive Disorder (MDD)

A. At least one of the following three abnormal moods which significantly interfered with the person's life:
   1. abnormal depressed mood most of the day, nearly every day, for at least 2 weeks;
   2. abnormal loss of all interest and pleasure most of the day, nearly every day, for at least 2 weeks; and
   3. if 18 or younger, abnormal irritable mood most of the day, nearly every day, for at least 2 weeks.

B. At least five of the following symptoms have been present during the same 2-week depressed period:
   1. abnormal depressed mood (or irritable mood if a child or adolescent);
   2. abnormal loss of all interest and pleasure;
   3. appetite or weight disturbance, either:
      - abnormal weight loss (when not dieting) or decrease in appetite;
      - abnormal weight gain or increase in appetite;
   4. sleep disturbance, either abnormal insomnia or abnormal hypersomnia;
   5. activity disturbance, either abnormal agitation or abnormal slowing (observable by others);
   6. abnormal fatigue or loss of energy;
   7. abnormal self-reproach or inappropriate guilt;
   8. abnormal poor concentration or indecisiveness; and
   9. abnormal morbid thoughts of death (not just fear of dying) or suicide.

C. The symptoms are not attributable to a mood-incongruent psychosis.

D. There has never been a manic episode, a mixed episode, or a hypomanic episode.

E. The symptoms are not attributable to physical illness, alcohol, medication, or street drugs.

F. The symptoms are not attributable to normal bereavement.

## BOX 12-2. Diagnostic and Statistical Manual of Mental Disorders, Text Revision Criteria for Bipolar Disorder (abbreviated)

### BIPOLAR I DISORDER

Single manic episode:

A. Presence of only one manic episode and no past major depressive episodes (Note: Recurrence is defined as either a change in polarity from depression or an interval of at least 2 months without manic symptoms.)

B. The manic episode is not better accounted for by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder not otherwise specified.

In cases where a patient has had multiple mood episodes, bipolar I subtypes are defined by the most recent mood episode:

- Most recent episode hypomanic
- Most recent episode manic
- Most recent episode mixed
- Most recent episode depressed
- Most recent episode unspecified

### BIPOLAR II DISORDER

A. Presence (or history) of one or more major depressive episodes

B. Presence (or history) of at least one hypomanic episode

C. There has never been a manic episode or a mixed episode

D. The mood episodes in criteria A and B are not better accounted for by schizoaffective disorder and are not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder not otherwise specified

E. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

## DIAGNOSTIC CRITERIA FOR AFFECTIVE DISORDERS

A **major depressive episode** is defined clinically by at least five of the following symptoms during the same 2-week period: persistently depressed mood or the loss of interest and pleasure in nearly all activities; weight change or significant change in appetite; insomnia or hypersomnia; psychomotor slowing or agitation; a sense of worthlessness or inappropriate guilt; fatigue or loss of energy; impaired concentration or indecisiveness; and recurrent thoughts of suicide or death. At least one of these symptoms must be depressed mood or loss of interest, and the symptoms must be severe enough to cause clinically significant distress or impairment of function. In the introductory case, Dr. Lee makes a clinical diagnosis of major depressive episode based on Mary having had essentially all these symptoms for more than a month.

A **manic episode** is defined clinically as an abnormally elevated, expansive, or irritable mood that lasts for at least 1 week and is accompanied by at least three of the following symptoms (or four, if mood is irritable): inflated self-esteem or grandiosity; decreased need for sleep; pressured speech (i.e., rapid, loud, emphatic, and unyielding speech); racing thoughts; distractibility; increased involvement in goal-directed activities or psychomotor agitation; and excessive involvement in pleasurable activities with high potential for painful consequences. To meet the criteria for a manic episode, these symptoms must cause impairment in functioning, require hospitalization, or be associated with psychotic symptoms.

When an episode lasts for 4 or more days and does not result in hospitalization or other adverse events, it is considered to be a **hypomanic episode** (literally, a "little mania"). In the introductory case, Mary had a hypomanic episode. If Dr. Lee had not intervened, her symptoms could have progressed to frank mania. When symptoms of a manic episode and a depressive episode are present simultaneously, it is referred to as a **mixed** episode. Major depressive, hypomanic, and mixed episodes form the building blocks for **major depressive disorder (MDD)** and **bipolar disorder (BPD).** MDD is characterized by episodes of depression that may be recurrent. BPD is characterized by episodes of mania or hypomania that may be interspersed with episodes of depression.

## THE MONOAMINE THEORY OF DEPRESSION

The biological basis for depression began to be understood when keen observers noticed that drugs in clinical trials for other diseases affected patients' moods. In the 1950s, the antihypertensive agent reserpine was noted to induce depression in 10%–15% of patients. Researchers then found that reserpine could induce depression in animal models as well as in humans. Reserpine depletes 5HT, NE, and DA in presynaptic neurons by inhibiting the transport of these neurotransmitters into synaptic vesicles. The drug binds irreversibly to the vesicular monoamine transporter and ultimately destroys the vesicles. The 5HT, NE, and DA that accumulate in the cytoplasm are degraded by mitochondrial MAO. The resulting decrease in monoamine neurotransmission is thought to be responsible for inducing a depressed mood.

Around the same time as the discovery of reserpine, two other drugs, **imipramine** and **iproniazid,** were found to elevate the moods of patients who were taking these drugs in clinical trials for schizophrenia and tuberculosis, respectively. Understanding the mechanisms of action of these two drugs subsequently led to the development of two of the most important classes of antidepressant medications, the **tricyclic antidepressants** (TCAs) and the **monoamine oxidase inhibitors** (MAOIs). In the late 1940s the tricyclic drug imipramine was developed for use in the treatment of psychotic patients, but it was subsequently noted to have strong antidepressant effects. Imipramine preferentially blocks 5HT transporters, and its active metabolite desipramine preferentially blocks NE transporters. Thus, neurotransmitters persist in the synapse at higher concentrations and for longer durations, yielding greater activation of postsynaptic 5HT and NE receptors. The antituberculosis drug iproniazid was shown to have antidepressant effects in 1951. Iproniazid inhibits monoamine oxidase and, thereby, prevents the degradation of 5HT, NE, and DA. The resulting increase in cytosolic neurotransmitter leads to increased neurotransmitter uptake into vesicles and, consequently, to greater release of neurotransmitter upon exocytosis.

The drug studies described above strongly suggested that the central monoaminergic serotonin and norepinephrine systems are intimately involved in the pathogenesis of depression. The **monoamine theory of depression** states that depression results from pathologically depressed levels of serotonin and/or norepinephrine neurotransmission. Based on this hypothesis, it follows that altering serotonin and/or norepinephrine neurotransmission could reverse depression. As a biologic disease related to long-term pathologic alterations in monoamine neurotransmission, MDD should, thus, be treatable by medications.

### Limitations of the Monoamine Theory

Although the classical antidepressants are pharmacologically active at their molecular and cellular sites of action almost immediately, their clinical antidepressant effects are generally not seen until 3 or more weeks of continuous treatment have elapsed. Similarly, although reserpine rapidly depletes neurotransmitter in monoaminergic systems, it takes several weeks of continued treatment with reserpine to induce depression. The unexplained delay in the onset of action of these drugs remains a central conundrum in understanding the pathophysiology of depression.

In some patients, drugs that selectively increase 5HT neurotransmission eliminate depression; whereas, drugs that selectively increase NE neurotransmission have little or no effect. In other patients, drugs affecting the NE system are more beneficial than those affecting the 5HT system. Overall, each individual drug is effective in about 70% of patients suffering from depression, and drugs that have markedly different efficacies in blocking the reuptake of NE and/or 5HT may have similar clinical effectiveness. These clinical observations, as with the delay in the therapeutic effect of antidepressants, are not easily explained by the monoamine theory.

### Role of Monoamine Receptor Sensitivity Changes in the Therapeutic Action of Antidepressants

Desensitization of presynaptic 5HT and/or NE autoreceptors may provide a partial explanation for the limitations of the monoamine theory of depression. Somewhat paradoxically, treatment with a classical antidepressant drug produces an immediate decrease in the frequency of neuronal firing in the locus ceruleus and/or the raphe nucleus (depending on the drug), with a concomitant decrease in the synthesis and release of 5HT and NE. This observation suggests that the antidepressant–induced increase in synaptic cleft neurotransmitter concentration results in acute feedback inhibition of neuronal firing via $5HT_{1D}$ and $\alpha_2$ autoreceptors for 5HT and NE, respectively. In response to the elevated levels of neurotransmitter, inhibitory autoreceptor stimulation acutely down-regulates the activity of the rate-limiting enzymes TPH and TH, and acutely reduces the rate of neuronal firing. The net effect is that short-term antidepressant medication does not significantly increase postsynaptic signaling.

In contrast, chronic use of antidepressants causes the inhibitory autoreceptors themselves to be down-regulated, leading to enhancement of neurotransmission. (Note that postsynaptic 5HT and NE receptors are also down-regulated by changes in neurotransmitter release and reuptake, but to a lesser degree.) Chronic, but not acute, treatment increases the levels of cAMP in target neurons, indicating that the net effect of chronic treatment with antidepressants is to increase second messenger signaling through the 5HT and/or NE pathways. The change in autoreceptor sensitivity takes several weeks to occur, consistent with the time-course of the therapeutic response in patients. Therefore, the delay in onset of the therapeutic response could be caused by physiologic inhibitory feedback mechanisms; only after chronic drug therapy, does gradual desensitization of autoreceptors allow increased neurotransmission (Fig. 12-4). Although speculative, this hypothesis regarding monoamine receptor sensitivity changes presents a credible explanation for the delay in onset of the therapeutic action of fluoxetine experienced by Mary.

## BIPOLAR DISORDER

In 1949, a researcher noted that lithium had a calming effect on animals and hypothesized that lithium would have a similar effect on manic patients. Subsequent studies proved this hypothesis correct. This discovery sparked intense research on the biochemical effects of lithium and the mechanisms by which this drug exerts its antimanic effects. Although research on lithium has provided some insights, the mechanisms responsible for its antimanic effects remain poorly understood (see below). At about the same time, it was noted that antidepressant medications can precipitate manic episodes in some patients with MDD. As with the mechanism of action of lithium, the mechanism by which antidepressant medications cause the switch from MDD to bipolar disorder is not understood.

Several decades later, in the 1970s, some researchers considered the possibility that mania could be related to epilepsy because both disorders exhibit episodic patterns involving brain overactivity. Subsequent research did not bear out this relationship, but such anticonvulsants as **carbamazepine** and **valproic acid** were found to have some efficacy in the treatment of BPD. Again, the mechanisms responsible for the clinical effectiveness of these agents remain unclear.

Today, lithium, carbamazepine, valproate, and certain other anticonvulsant drugs (see Chapter 13, Pharmacology of Abnormal Electrical Neurotransmission in the Central Nervous System) are used to treat mania and bipolar depression and to prevent future episodes of mood disorder. Antipsychotics are sometimes used in the treatment of acute mania, although their mechanism of action in BPD is also poorly understood.

## PHARMACOLOGIC CLASSES AND AGENTS

Four classes of drugs are commonly used to treat affective disorders. Tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs) are all used in the treatment of depression and major depressive disorder. A heterogeneous group of newer heterocyclic amine compounds, referred to as "atypical antidepressants," are also effective for the treatment of depression. Additionally, lithium is commonly prescribed for the treatment of mania.

## TRICYCLIC ANTIDEPRESSANTS (TCAs)

The TCAs derive their name from their common chemical backbone, consisting of three rings that include two aromatic rings attached to a cycloheptane ring. The prototype TCA is **imipramine**. The TCAs share pharmacologic properties, depending on the structure of their amine: TCAs with secondary amines preferentially affect the NE system; whereas, those with tertiary amines primarily affect the 5HT system.

### Mechanism of Action

TCAs inhibit the reuptake of 5HT and NE from the synaptic cleft by blocking 5HT and NE reuptake transporters, respectively. These agents do not affect DA reuptake (Fig. 12-5). The molecular mechanism of transporter inhibition remains to be elucidated. It is believed that the prolongation of time spent by neurotransmitter in the synaptic cleft results in increased receptor activation and, eventually, in enhancement of postsynaptic response (see above). Despite widely varying affinities for 5HT and NE reuptake transporters, the TCAs are markedly similar in their clinical efficacy.

### Side Effects

The broad side effect profile of TCAs results from their ability to bind a wide array of channels and receptors in addition

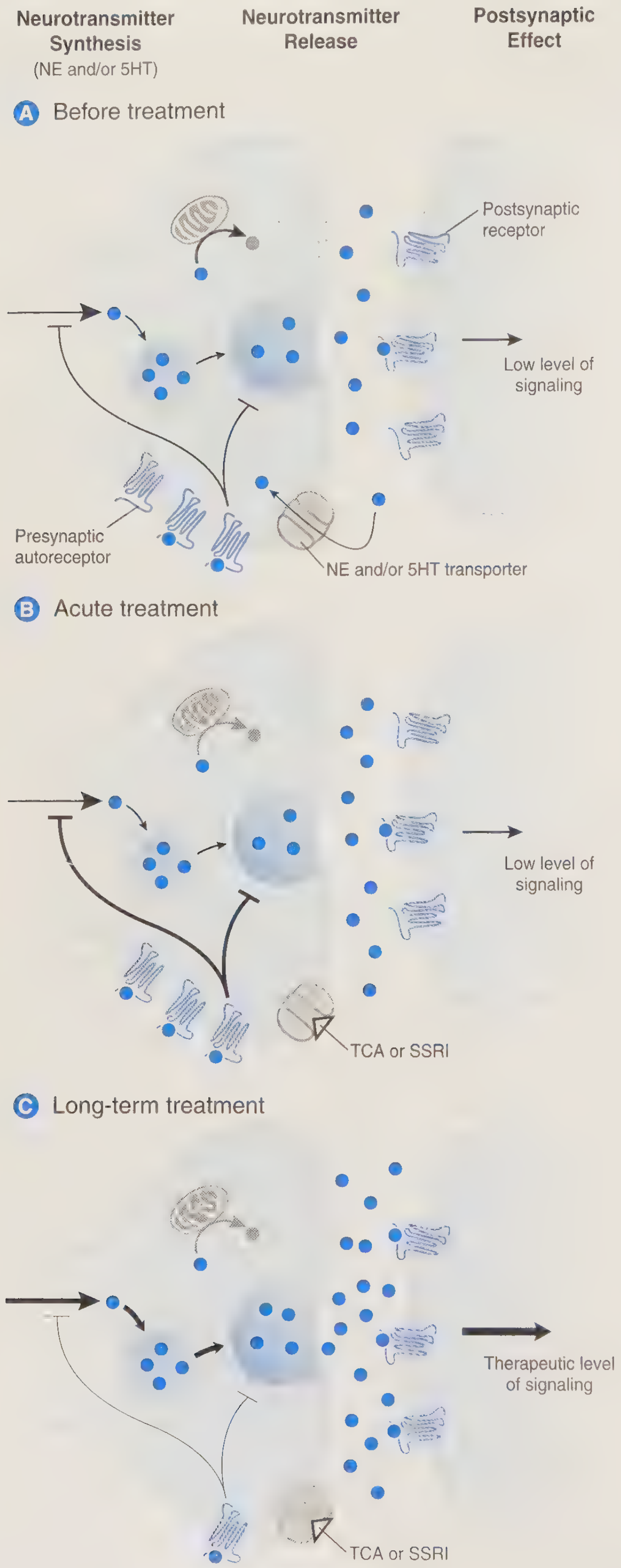


**Figure 12-4. Postulated Mechanism of the Delay in Onset of the Therapeutic Effect of Antidepressant Medications.** **A.** Before treatment, neurotransmitters are released at pathologically low levels and exert steady-state levels of autoinhibitory feedback. The net effect is a pathologically low baseline level of postsynaptic receptor activity (signaling). **B.** Short-term use of antidepressant medication results in increased release of neurotransmitter and/or increased duration of neurotransmitter action in the synaptic cleft. Both effects cause increased stimulation of inhibitory autoreceptors, with increased inhibition of neurotransmitter synthesis and increased inhibition of exocytosis. The net effect is to dampen the initial effect of the medication, and postsynaptic receptor activity remains at pretreatment levels. **C.** Chronic use of antidepressant medication results in desensitization of the presynaptic autoreceptors. Thus, the inhibition of neurotransmitter synthesis and exocytosis is reduced. The net effect is enhanced postsynaptic receptor activity, leading to a therapeutic response. NE, norepinephrine. 5HT, serotonin. TCA, tricyclic antidepressant. SSRI, selective serotonin reuptake inhibitor.

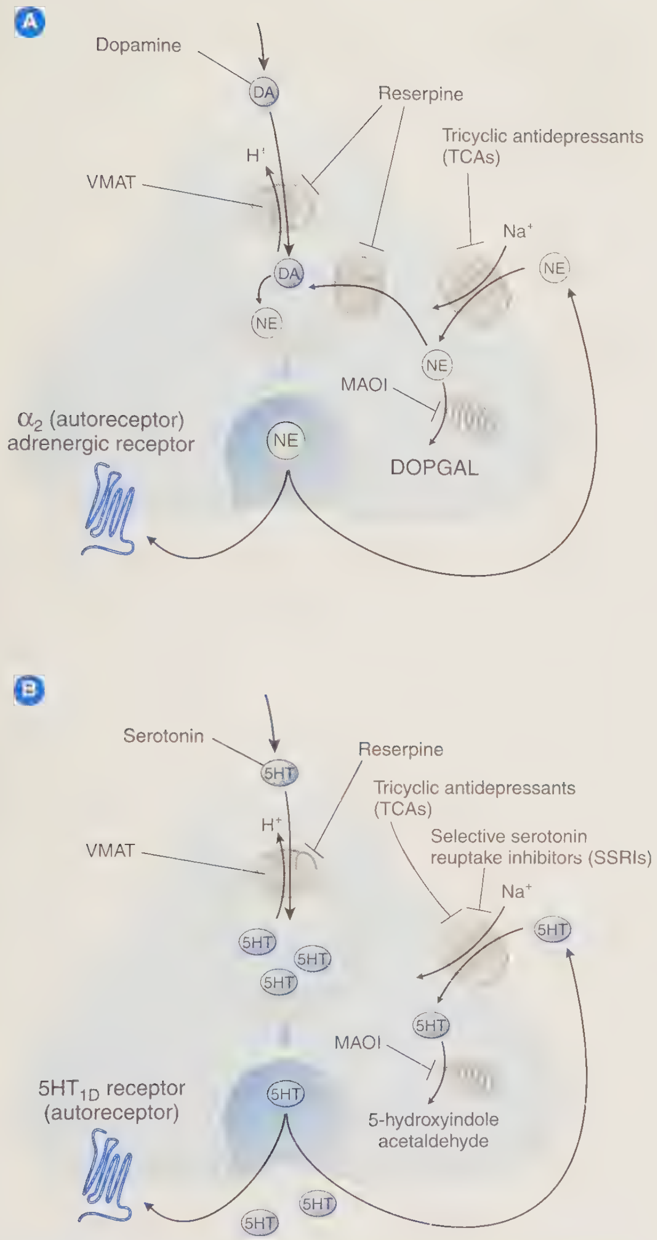

A
Dopamine
DA
Reserpine
Tricyclic antidepressants (TCAs)
H+
VMAT
Na+
NE
DA
NE
NE
MAOI
$\alpha_2$ (autoreceptor) adrenergic receptor
NE
DOPGAL
B
Serotonin
5HT
Reserpine
Tricyclic antidepressants (TCAs)
H+
Selective serotonin reuptake inhibitors (SSRIs)
VMAT
Na+
5HT
5HT
5HT
5HT
5HT
MAOI
$5HT_{1D}$ receptor (autoreceptor)
5HT
5-hydroxyindole acetaldehyde
5HT
5HT

**Figure 12-5. Sites and Mechanisms of Action of Antidepressant Drugs.** The sites of action of antidepressant drugs and of reserpine (which can induce depression) are indicated in noradrenergic neurons **(A)** and serotonergic neurons **(B).** Monoamine oxidase inhibitors (MAOIs) inhibit the mitochondrial enzyme monoamine oxidase (MAO); the resulting increase in cytosolic monoamines leads to increased vesicular uptake of neurotransmitter and to increased release of neurotransmitter during exocytosis. Tricyclic antidepressants (TCAs) and heterocyclic antidepressants inhibit both the norepinephrine transporter (NET) and the serotonin transporter (5HTT), causing increased levels of both NE and 5HT in the synaptic cleft. Selective serotonin reuptake inhibitors (SSRIs) specifically inhibit the 5HTT-mediated reuptake of 5HT. TCAs, heterocyclics, and SSRIs increase the duration of neurotransmitter action in the synaptic cleft, leading to increased downstream signaling. Reserpine, which can induce depression in humans and in animal models, blocks the VMAT-mediated uptake of monoamines into synaptic vesicles, which ultimately destroys the vesicles.

to their therapeutic targets, the 5HT and NE reuptake transporters. The most dangerous side effects of TCAs involve the cardiovascular system. TCAs are hypothesized to affect sodium channels in a quinidine-like manner. The **quinidine-like side effects of TCAs** include potentially lethal conduction delays, such as first-degree atrioventricular and bundle branch blocks.

TCAs can also act as antagonists at muscarinic (cholinergic), histamine, adrenergic, and dopamine receptors. The *anticholinergic effects* are most prominent and include symptoms typical of muscarinic acetylcholine receptor blockade: nausea, vomiting, anorexia, dry mouth, blurred vision, confusion, constipation, tachycardia, and urinary retention. The *antihistaminergic effects* include sedation and weight gain, and the *antiadrenergic effects* include orthostatic hypotension, reflex tachycardia, drowsiness, and dizziness. Orthostatic hypotension is an especially significant risk for elderly patients, and TCA use must be monitored carefully in such patients. Finally, TCAs may also precipitate mania in bipolar patients.

Like their widely varying affinities for the NE and/or 5HT reuptake transporters, the affinities of the various TCAs for the muscarinic, histamine, adrenergic, and dopamine receptors also vary. Because the therapeutic efficacy is similar among the TCAs, despite their varying affinities for the reuptake transporters, their relative affinities for the other receptors determine the profile of adverse reactions for each TCA. Clinically, the choice of a particular TCA is commonly based on the relative tolerability of the drug's side effect profile.

### Pharmacokinetics

TCAs are lipophilic molecules that bind avidly to plasma proteins and to tissues. The TCAs are well absorbed via the gastrointestinal tract and undergo highly variable first-pass metabolism. Many of the primary metabolites are pharmacologically active. These metabolites are products of demethylation or hydroxylation of the primary drug via the P450 enzyme system (primarily by CYP2D6). Because of the wide variability among individuals in metabolizing these drugs, the dosage of TCAs must be determined on an individual basis. The TCAs are inactivated by glucuronidation and eliminated by renal clearance.

## SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)

The treatment of depression was revolutionized with the introduction of **selective serotonin reuptake inhibitors** (SSRIs) in 1987. The first SSRI to be introduced was fluoxetine; this drug is still one of the most widely prescribed SSRIs. Other SSRIs include **citalopram, fluvoxamine, paroxetine,** and **sertraline.** Although the effectiveness of the SSRIs is similar to that of the TCAs for the treatment of depression, their higher selectivity and reduced side effect profile has made them first-line agents for the treatment of depression. Although SSRIs are generally more costly than TCAs, they are associated with fewer treatment failures than TCAs because the TCAs are often abandoned on account of their intolerable side effects. SSRIs are also used to treat panic disorder and obsessive compulsive disorder.

## Mechanism of Action

The SSRIs are similar to the TCAs in their mechanism of action, except that the SSRIs are significantly more selective for 5HT transporters (Fig. 12-5B). Inhibition of serotonin reuptake increases synaptic serotonin levels, causing increased 5HT receptor activation and enhanced postsynaptic responses. At low doses, SSRIs are believed to bind primarily to 5HT transporters; whereas, at higher doses, they lose selectivity and also bind NE transporters. Despite widely varying chemical structures, the SSRIs have clinical efficacies similar to the TCAs and to one another. Thus, the choice of drug often depends on such issues as cost and tolerability of side effects. In addition, because of the variability of individual patient responses to individual antidepressants, finding the right drug often requires some trial and error.

## Side Effects

Because the SSRIs are more selective than the TCAs at clinically effective doses, they have far fewer adverse effects. SSRIs lack significant cardiotoxicity, and do not bind as avidly to muscarinic (cholinergic), histamine, adrenergic, or dopamine receptors. As a consequence, SSRIs are generally better tolerated than TCAs, and have become the first-line agents for treatment of MDD. The enhanced selectivity of the SSRIs also means that these agents have a larger therapeutic index than the TCAs. This is an important consideration in the depressed patient, who may attempt to commit suicide by intentionally overdosing on his or her medication.

The SSRIs are not entirely without side effects, however. All SSRIs can cause some degree of sexual dysfunction. Another common adverse effect is gastrointestinal distress; sertraline is more often associated with diarrhea, while paroxetine is associated with constipation. A more serious adverse effect of the SSRIs is **serotonin syndrome,** a rare but dangerous elevation of 5HT that can occur when both an SSRI and a monoamine oxidase inhibitor (MAOI; see below) are administered simultaneously. The clinical manifestations of serotonin syndrome include hyperthermia, muscle rigidity, myoclonus, and rapid fluctuations in mental status and vital signs. Finally, as with TCAs and MAOIs, SSRIs can cause a "switch" from depression to mania or hypomania in vulnerable patients. The fluoxetine that Mary was prescribed for MDD was likely responsible for her subsequent manic episode, although in any individual patient, it is difficult to attribute unambiguously an incidence of mania to the action of an antidepressant. The mechanism of the SSRI-induced switch from depression to mania or hypomania is currently unknown.

## Pharmacokinetics

The SSRIs are similar to the TCAs with respect to their pharmacokinetics. As with most drugs acting in the CNS, SSRIs are lipophilic and metabolized in the liver. Most SSRIs inhibit the cytochrome P450 2D6 isoenzyme; this inhibition may affect the metabolism of other drugs that are also acted on by CYP2D6, including other antidepressants, certain antipsychotics, benzodiazepines, such anticonvulsants as phenytoin and carbamazepine, warfarin, β-blockers, type IC antiarrhythmics, and some chemotherapeutic agents. Most SSRIs have half-lives on the order of 24 hours, making them ideal for once a day dosing. Fluoxetine has recently become available in a sustained release formulation that allows weekly dosing.

# MAO INHIBITORS (MAOIs)

MAOIs are classified according to their specificity for the MAO-A and MAO-B isoenzymes and according to the reversibility or irreversibility of their binding. The older MAOIs are nonselective, and most older MAOIs, such as **iproniazid, phenelzine,** and **isocarboxazid,** are irreversible inhibitors. Newer MAOIs, such as **moclobemide, befloxatone,** and **brofaromine,** are selective for MAO-A and bind reversibly, and are thus called **reversible inhibitors of monoamine oxidase A** (RIMAs). MAO-B selective inhibitors, such as **selegiline** (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission), are less effective for treatment of depression.

## Mechanism of Action

MAOIs block the deamination of monoamines by binding to and inhibiting the functional flavin moiety of MAO (Fig. 12-5). By inhibiting the degradation of monoamines, MAOIs increase the 5HT and NE available in the cytoplasm of the presynaptic neuron for neurotransmission. The increase in cytoplasmic levels of these monoamines leads not only to increased uptake and storage of 5HT and NE in synaptic vesicles, but also to some constitutive leakage of the monoamines into the synaptic cleft.

## Side Effects

The most toxic side effect of MAOI use is systemic **tyramine toxicity.** Because gastrointestinal and hepatic MAO metabolizes tyramine, consumption of foods that contain tyramine, such as aged cheese and red wine, can lead to excess levels of circulating tyramine. Tyramine is an indirect sympathomimetic that can stimulate the release of large amounts of stored catecholamines by reversing the reuptake transporters (see Chapter 8). This uncontrolled catecholamine release can induce a *hypertensive crisis* characterized by headache, tachycardia, nausea, cardiac arrhythmia, and stroke. The older MAOIs are no longer considered first-line therapy for depression because of the potential for systemic tyramine toxicity. The newer MAOIs (i.e., the RIMAs, which bind reversibly to MAO), are displaced by high concentrations of tyramine, resulting in significantly more tyramine metabolism and, hence, less tyramine toxicity. MAOIs, as can other antidepressants, can precipitate manic or hypomanic episodes in some bipolar patients.

## Pharmacokinetics

All antidepressant drugs, including MAOIs, are hydrophobic and cross the blood–brain barrier (see Chapter 6). They are well absorbed orally and are metabolized to active metabolites in the liver. These metabolites are subsequently inactivated by acetylation, also in the liver. Excretion is primarily via renal clearance. The older, irreversibly binding MAOIs are cleared from the circulation as complexes with MAO, and are effectively inactivated only when a new enzyme is synthesized.

## OTHER ANTIDEPRESSANTS

Other antidepressants, which are sometimes referred to as "atypical antidepressants," include **bupropion, mirtazapine, nefazodone, trazodone,** and **venlafaxine.** They are categorized together here only because they do not fit conveniently into other categories. These agents are newer than TCAs and act by several different mechanisms, although some have unknown or incompletely characterized mechanisms of action. Bupropion seems to act mechanistically like the amphetamines (see Chapter 8). Mirtazapine blocks $5HT_{2A}$, $5HT_{2C}$, and the $\alpha_2$-adrenergic autoreceptor, and presumably decreases neurotransmission at $5HT_2$ receptors while increasing NE neurotransmission. Nefazodone and trazodone also block postsynaptic $5HT_2$ receptors. Venlafaxine blocks the 5HT reuptake transporter and the NE reuptake transporter in a concentration-dependent manner; at lower concentrations, it behaves as an SSRI. Overall, these atypical antidepressants have relatively few side effects and demonstrate similar clinical efficacies despite their widely heterogeneous mechanisms of action and molecular targets.

## LITHIUM

Lithium, commonly administered as lithium carbonate, is a small monovalent cation that is similar in electrochemical properties to sodium and potassium. At therapeutic concentrations, lithium enters cells via $Na^+$ channels. Because lithium can mimic other small monovalent cations, it has the potential to disrupt any number of proteins and transporters that require specific cation cofactors.

### Mechanism of Action

Lithium exerts numerous effects at the cellular level. Its effect on the regeneration of inositol for second messenger signaling is particularly well studied, although this effect is not necessarily central to its therapeutic actions. In the inositol pathway, $\alpha$-adrenergic, some muscarinic, and $5HT_2$ receptors are G protein-coupled receptors that activate phospholipase C (PLC), which cleaves phosphatidylinositol 4,5-bisphosphate ($PIP_2$) to the signaling molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$). $IP_3$ signaling is terminated by conversion to inositol 4,5-bisphosphate ($IP_2$), either directly or via an $IP_4$ intermediate. $Li^+$ inhibits both the inositol phosphatase that dephosphorylates $IP_2$ to inositol phosphate ($IP_1$), and the inositol phophatase that dephosphorylates $IP_1$ to free inositol. Because free inositol is essential for the regeneration of $PIP_2$, lithium effectively blocks the phosphatidylinositol signaling cascade in the brain. (Inositol circulates freely in blood, but cannot pass the blood–brain barrier. The two mechanisms of inositol synthesis in CNS neurons, regeneration from $IP_3$ and de novo synthesis from glucose-6-phosphate, are both inhibited by lithium.) By blocking the regeneration of $PIP_2$, lithium inhibits central adrenergic, muscarinic, and serotonergic neurotransmission via $IP_3$ and DAG.

Disruption of the phosphatidylinositol signaling cascade was previously thought to be the primary mechanism of lithium's mood-stabilizing action. However, recent studies suggest that other actions of lithium may also be relevant. These actions include: increasing 5HT neurotransmission by increasing neurotransmitter synthesis and release; decreasing NE and DA neurotransmission by inhibiting neurotransmitter synthesis, storage, release, and reuptake; inhibiting adenylyl cyclase by decoupling G proteins from neurotransmitter receptors; and altering electrochemical gradients across cell membranes by substituting for $Na^+$ and/or blocking $K^+$ channels. Possible neurotrophic effects of lithium are also under investigation.

### Side Effects

Lithium has a narrow therapeutic window and a wide range of side effects, leading patients such as Mary to be concerned about its potential adverse effects. **Acute lithium intoxication,** a clinical syndrome marked by nausea, vomiting, diarrhea, renal failure, neuromuscular dysfunction, ataxia, tremor, confusion, delirium, and seizures, is a medical emergency. Hyponatremia—for example, caused by low dietary sodium intake or some diuretics—can lead to increased lithium reabsorption in the proximal tubules and elevation of serum lithium to toxic levels.

Flattening or inversion of T waves on the ECG may result from lithium's inhibition of $K^+$ channels on cardiac myocytes. Inhibition of $K^+$ entry into myocytes leads to abnormalities in repolarization, resulting in the abnormal T waves. In addition, the transmembrane electrical potential is shifted because inhibition of $K^+$ entry into cells leads to extracellular hyperkalemia and intracellular hypokalemia. This shift in transmembrane electrical potential exposes patients to a greater risk of sudden cardiac arrest from small changes in potassium balance.

Antidiuretic hormone and thyroid-stimulating hormone both activate adenylyl cyclase, which is inhibited by lithium. By this mechanism, lithium treatment can also lead to a decrease in urine concentrating ability (and, in a significant minority of cases, **nephrogenic diabetes insipidus**) and to hypothyroidism and/or goiter.

Given the wide range of side effects that may accompany lithium treatment and the euphoria that may be associated with manic or hypomanic episodes, many patients are hesitant to begin such treatment. However, lithium and a limited number of other mood-stabilizing drugs (see Drug Summary Table) help to prevent depressive episodes as well as mania. Mood-stabilizing medications may allow the patient to regain control of his or her life by lessening the impact of both depressive and manic episodes. For example, some patients are incapable of recognizing the detrimental effects of their actions while they are manic, such as the financial consequences of a major spending spree.

## Pharmacokinetics

Lithium is well absorbed in the gastrointestinal tract and distributes as a cation in total body water. It is excreted unchanged by filtration in the kidneys. It is absorbed in both proximal tubules, where it competes with $Na^+$ reabsorption, and the loop of Henle, where it does not compete with $Na^+$ reabsorption. Lithium is not absorbed in the distal tubules.

## *Conclusion and Future Directions*

This chapter has discussed MDD and BPD, which are clinically defined syndromes that respond well to pharmacologic treatment. Neither disease is completely understood at the molecular and cellular levels, however. The monoamine theory of depression forms a framework for considering the pathophysiology and treatment of MDD, although this theory has inconsistencies that require further study. Therapy with drugs that increase synaptic concentrations of 5HT and NE are effective in many cases of MDD and form the basis of treatment for this disorder. The long delay between initiation of treatment and onset of clinical improvement may occur because of slow changes in receptor sensitivities. TCAs, SSRIs, MAOIs, and other antidepressants have similar clinical efficacies, although patients may respond to one drug and not to another. TCAs nonselectively inhibit 5HT and NE reuptake transporters; SSRIs selectively block 5HT transporters; and MAOIs inhibit the degradation of both 5HT and NE. The choice of antidepressant medication for an individual patient depends on the two goals of finding an effective agent for that patient, often by trial and error, and minimizing side effects. SSRIs are the most commonly prescribed antidepressants because of their favorable therapeutic index.

BPD is less well understood than MDD, in terms of both its pathophysiology and the mechanisms underlying effective therapies. Agents used to treat BPD include lithium, valproate and carbamazepine; in some circumstances, antidepressants and antipsychotics are also used. These drugs are sometimes called “mood stabilizers” because they limit the extremes of mania and depression. Unfortunately, their mechanism of action is not well understood.

Recent advances in drug development for the treatment of MDD have focused on a deeper understanding of the current drugs and the physiology of their molecular targets. Currently approved antidepressants are administered as racemic mixtures, and the isolation of active stereoisomers, such as S-citalopram, may yield drugs with better tolerability. Pharmacogenomic approaches have uncovered polymorphisms in the 5HT reuptake transporter that could affect the likelihood of an individual’s response to SSRI treatment. Thus, pharmacogenomics may lead to better matching of drugs to patients by identifying patients who are particularly likely or particularly unlikely to respond to or tolerate a specific medication. Other drug targets beyond the monoamine systems are also promising, including neuropeptide antagonists of substance P and CRH.

In another new and exciting development, the increased stimulation of receptors in treated patients has been shown to elevate levels of cAMP and induce such transcription factors as cAMP response element binding protein (CREB) in the hippocampus. In fact, neurogenesis seems to be reduced in the hippocampi of some MDD patients. The possible link between neural plasticity and depression is a topic of current investigation and potentially a new therapeutic target.

## *Suggested Readings*

Arane GW, Hyman SE, Rosenbaum JF. Handbook of Psychiatric Drug Therapy. 4th Ed. Philadelphia: Lippincott Williams & Wilkins, 2000. (*Psychiatric handbook that emphasizes the molecular understanding of psychiatric diseases, including MDD and bipolar disorder, and of the drugs used to treat those diseases.*)

Price LH, Heninger GR. Lithium in the treatment of mood disorders. N Engl J Med 1994;331:591–594. (*Review of lithium and its possible mechanisms of action in bipolar disorder.*)

Richelson, E. Pharmacology of antidepressants. Mayo Clin Proc 2001;76:511–527. (*Broad and thorough overview of the molecular mechanisms and cellular targets of current antidepressant medications.*)

Santarelli L, Saxe M, Gross C, Surget A, et al. Requirement of hippocanpal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–809. (*Recent research article on MDD.*)

Tkacher D, Mimmack ML, Ryan MM, Wayland M, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 2003;362:798–805. (*Recent research article in BPD.*)

## Drug Summary Table Pharmacology of Affective Disorders

| Drug | [illegible] | [illegible] | [illegible] | [illegible] |
|---|---|---|---|---|
| **TRICYCLIC ANTIDEPRESSANTS (TCAs)**<br>*Mechanism—Block 5HT and NE reuptake transporters (5HTT and NET)* | | | | |
| **Amitriptyline**<br>**Clomipramine**<br>**Desipramine**<br>**Doxepine**<br>**Imipramine**<br>**Nortriptyline**<br>**Protriptyline**<br>**Trimipramine** | All TCAs can be used in:<br>Major depressive disorder<br>Anxiety disorders<br>Chronic pain | All TCAs can cause:<br>Anticholinergic effects<br>Orthostatic hypotention<br>Quinidine-like cardiac toxicity<br>Sedation<br>Can precipitate mania in a fraction of patients | | Many TCAs have active metabolites |
| **SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)**<br>*Mechanism—Selectively block 5HT reuptake transporter* | | | | |
| **Citalopram**<br>**Fluoxetine**<br>**Fluvoxamine**<br>**Paroxetine**<br>**Sertraline** | All SSRIs can be used in:<br>Major depressive disorder<br>Anxiety disorders | All SSRIs can cause:<br>Sexual dysfunction<br>Serotonin syndrome, in conjunction with MAOI use<br>Can precipitate mania in a fraction of patients | SSRIs can have drug–drug interactions via cytochrome P450<br>SSRIs can cause serotonin syndrome, in conjunction with MAOI use | SSRIs are similar in efficacy to TCAs, with fewer side effects |
| **MONOAMINE OXIDASE INHIBITORS (MAOIs)**<br>*Mechanism—Inhibit cytoplasmic degradation of 5HT and NE by MAO* | | | | |
| *Irreversible block:*<br>**Phenelzine**<br>**Tranylcypromine**<br>*Reversible block (RIMA):*<br>**Befloxatone**<br>**Brofaromine**<br>**Moclobemide** | All MAOIs can be used in:<br>Major depressive disorder<br>Anxiety disorders | Irreversible MAOIs can cause hypertensive crisis due to tyramine toxicity | | Irreversible MAOIs require tyramine-free diet<br>MAOIs, both irreversible and reversible, are not first line antidepressant agents<br>Reversible MAOIs (RIMAs) have less tyramine toxicity, because high levels of tyramine can displace the reversible RIMA from MAO |
| **OTHER ANTIDEPRESSANTS**<br>*Mechanism—Various, including postsynaptic 5HT receptor blockade, stimulation of neurotransmitter release, and selective blockade of NE reuptake transporter* | | | | |
| **Buproprion**<br>**Mirtazapine**<br>**Nefazodone**<br>**Trazodone**<br>**Venlafaxine** | Other antidepressants can be used in:<br>Major depressive disorder<br>Anxiety disorders | Other antidepressants have various side effects (see text) | | Other antidepressants are similar in efficacy to TCAs, with fewer side effects |

*(Continued)*

**Drug Summary Table** **Pharmacology of Affective Disorders** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicity | Interactions |
|---|---|---|---|
| **LITHIUM**<br>*Mechanism—Unknown* | | | |
| **Lithium** | Bipolar disorder | Acute lithium intoxication<br>Nephrogenic diabetes insipidis<br>Hypothyroidism | Some diuretics may elevate plasma lithium concentrations |
| **ANTICONVULSANTS**<br>*Mechanism—Unknown (for treatment of bipolar disorder)* | | | |
| **Valproate**<br>**Carbamazepine**<br>**Lamotrigene** | All of these anticonvulsants can be used in bipolar disorder | See Chapter 13 | |

# 13

# Pharmacology of Abnormal Electrical Neurotransmission in the Central Nervous System

Edmund A. Griffin, Jr. and Daniel H. Lowenstein

**Introduction**
**Case**
**Physiology**
**Pathophysiology**
  Pathophysiology of Partial Seizures
  Pathophysiology of Secondary Generalized Seizures
  Pathophysiology of Primary Generalized Seizures
**Pharmacologic Classes and Agents**
  Drugs That Enhance Na+ Channel-Mediated Inhibition
    *Phenytoin*
    *Carbamazepine*
    *Lamotrigine*
  Drugs That Act at the T-type Calcium Channel
    *Ethosuximide*
    *Valproic Acid*
  Drugs That Enhance GABA-Mediated Inhibition
    *Gabapentin*
    *Benzodiazepines (Diazepam, Clonazepam)*
    *Barbiturates (Phenobarbital)*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

With over 10 billion neurons and an estimated $10^{13}$ synaptic connections, the human brain boasts unparalleled electrical complexity. Unlike myocardial tissue, where electrical signals spread synchronously through a syncytium of cells, proper functioning of the brain requires distinct isolation of electrical signals, and, thus, demands a far higher level of regulation. As seen below, control of this complex function begins at the level of the ion channel and is further maintained through the effects of these ion channels on the activity of highly organized neuronal networks. Abnormal function of ion channels and neural networks can result in rapid, synchronous, and uncontrolled spread of electrical activity, which is the basis of a **seizure.**

The seizure disorders belong to a heterogeneous group comprising a variety of clinical presentations and many different causes. They represent clinical manifestations of abnormal electrical activity in the brain and should be distinguished from **epilepsy,** which refers to the condition where an individual has a tendency toward recurrent seizures (i.e., a patient who has had a single seizure does not necessarily have epilepsy). Depending on the location of seizure activity in the CNS, a patient may experience a variety of symptoms. These symptoms include the relatively common, prominent motor symptoms and loss of consciousness seen in tonic–clonic seizures, as well as paroxysmal changes observed in a variety of nonmotor functions such as sensation, olfaction, vision, and higher-order functions, such as emotion, memory, language, and insight.

This chapter explores the molecular mechanisms by which the brain maintains precise control over the spread of electrical activity and how various abnormalities can undermine these physiologic mechanisms and lead to seizures. The various classes of antiepileptic drugs are then discussed, with an emphasis on molecular mechanisms for restoring inhibitory function in the brain and suppressing seizure activity.

## Case

Jon arrives in the emergency room with his brother Rob at 9:12 PM. Because his brother is still too lethargic to speak, Jon relays most of the story to the attending neurologist. The two had been watching television when Jon noticed that his 40-year-old brother seemed to be daydreaming. Never missing an opportunity to tease, Jon began chiding his brother for "spacing out." But instead of the boisterous laugh that he was so used to, Jon observed only a confused, almost fearful stare.

Jon recalls that, almost suddenly, his brother's right hand began to bend into an awkward position and then to shake. The shaking grew worse, progressing gradually from the hand to the arm and then to the entire right side of the body. Jon then noticed that Rob's body stiffened, almost as if he were attempting to contract every muscle in his body. This sustained contraction lasted for about 15 seconds, and was followed by shaking movements of all four limbs that lasted another 30 seconds or so. The frequency of the shaking slowed after several minutes, and Rob then became limp, began breathing very heavily, and remained unresponsive. Rob regained consciousness on the way to the emergency department.

At the hospital, an MRI scan shows a small neoplasm in his left temporal lobe. Because the neoplasm appears to be benign, Rob, following the advice of his physician, decides not to undergo surgery. The potential benefits and risks of various anticonvulsant drugs, including phenytoin, carbamazepine, valproate, and lamotrigine, are discussed, and it is decided to start Rob on a regimen of carbamazepine to prevent further seizures.

## QUESTIONS

1. By what mechanisms can a focal neoplasm result in a seizure?
2. Is there any clinical significance to the fearful, blank stare?
3. What is the significance of the order of the spread of the seizure from the hands, to the arm, and then to the leg?
4. The generalized seizure that followed the right-sided shaking included a tonic phase (stiffening) followed by a clonic phase (shaking). What occurs at the molecular level to cause these symptoms?
5. How do drugs such as phenytoin, carbamazepine, valproate, and lamotrigine prevent seizures? Why was carbamazepine chosen for Rob?

## PHYSIOLOGY

The normal human brain, in the absence of any lesions or genetic abnormalities, is capable of undergoing a seizure. Acute changes in the availability of excitatory neurotransmitters (e.g., caused by ingestion of the toxin **domoic acid**) or changes in the effect of inhibitory neurotransmitters (e.g., caused by injection of **penicillin,** a GABA antagonist) can result in massive seizure activity in the otherwise healthy human brain. These examples demonstrate that the complex circuits within the brain exist in a balance between excitatory and inhibitory factors, and that changes in either of these control mechanisms can lead to major dysfunction.

In the CNS, two important elements normally involved in the fine-tuning of neuronal signaling also function to prevent the repetitious and synchronous firing characteristic of a seizure. At the cellular level, a "refractory period" induced by Na$^+$ channel inactivation and K$^+$ channel-mediated hyperpolarization prevents abnormal repetitive firing in neuronal cells. As discussed previously (Chapter 5, Principles of Cellular Excitability and Electrochemical Transmission), action potentials are propagated by voltage-sensitive ion channels. After initiation in the axon hillock, the action potential is propagated by alternating currents of depolarizing Na$^+$ influx and hyperpolarizing K$^+$ efflux. Throughout the course of an action potential (Fig. 13-1), the Na$^+$ channels exist in three distinct states: (1) a **closed state** before activation, (2) an **open state** during depolarization, and (3) an **inactivated state** shortly after the peak of depolarization. Because Na$^+$ channels adopt the inactive state in response to depolarization, action potentials are intrinsically self-limiting—Na$^+$ channels will not recover from the inactive state until the membrane is sufficiently repolarized. K$^+$ channel opening repolarizes the cell, but the high K$^+$ efflux transiently hyperpolarizes the membrane beyond its resting potential, further increasing the time before a new action potential can be generated. Thus, *the biochemical properties of K$^+$ and Na$^+$ channels, under physiologic conditions, impose a limit on the frequency of firing, helping to prevent the repetitive firing characteristic of many seizure types.*

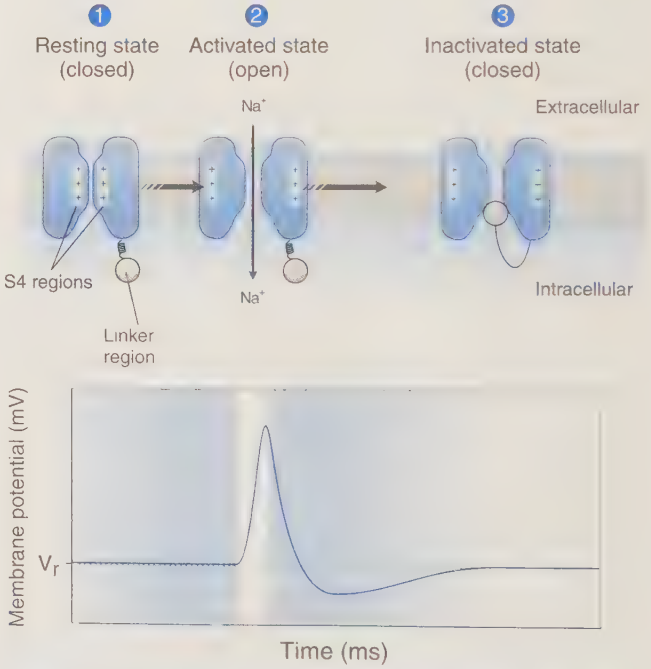


**Figure 13-1. Duration and Frequency of the Action Potential is Limited by Properties Intrinsic to the Sodium Channel.** The voltage-sensitive Na$^+$ channel exists in three different conformations during the course of an action potential. After opening transiently in response to membrane depolarization (2), the Na$^+$ channel spontaneously inactivates (3). This closure of the channel decreases the strength of the Na$^+$-mediated depolarization. Na$^+$ channels recover from inactivation only when the membrane potential is restored to its resting level ($V_r$). Membrane depolarization also has the effect of opening voltage-sensitive K$^+$ channels, which hyperpolarize the cell. Under hyperpolarizing conditions, the Na$^+$ channel adopts its resting (closed) conformation (1). During these refractory periods of Na$^+$ channel inactivation and membrane hyperpolarization, the neuron is essentially insensitive to depolarizing signals (see also Fig. 9-8).

Beyond the single cell level, **neural networks** ensure the specificity of neuronal signaling by restricting the effects of a given action potential to a defined area. Even a strong train of action potentials, if contained within about 1,000 neurons, will not generate seizure activity. This is quite a remarkable feat, given the close proximity of neurons in the CNS and the fact that a single neuron in the neocortex may have far more than 1,000 postsynaptic connections. As seen in the simplified neural network in Fig. 13-2, the firing neuron activates immediately neighboring neurons in addition to interneurons that transmit inhibitory (**GABA**) signals to surrounding neurons. This contrast of local amplification and surrounding cell inhibition results in what is referred to as **"surround inhibition."** Surround inhibition is essential to the normal function of the nervous system, because this phenomenon not only amplifies local signals but also provides insulation and protection against synchronicity in surrounding areas. Many seizure disorders seem to result from disruption of this intricate balance.

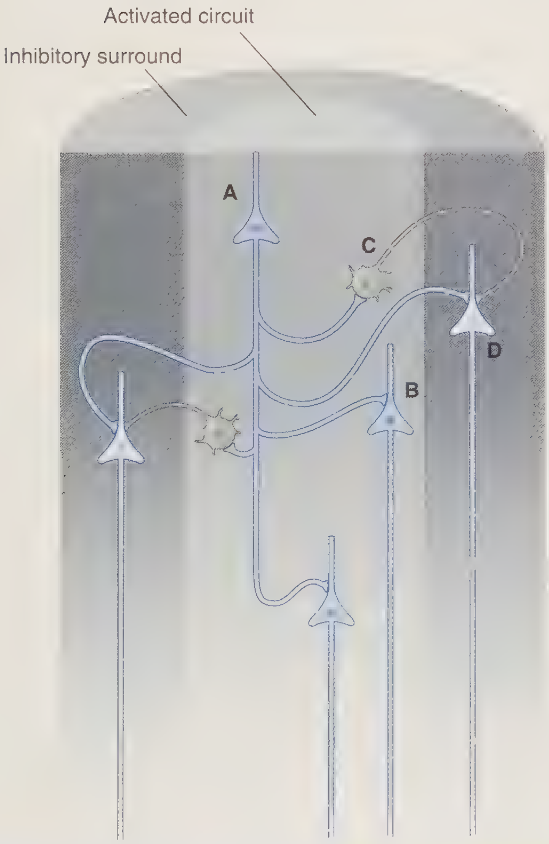


**Figure 13-2. Surround Inhibition Prevents Synchronization of Adjacent Neurons.** In this simplified neuronal circuit, neuron **A** sends excitatory projections (blue) to proximal neurons such as **B**. In addition to activating nearby neurons, cell **A** also activates GABAergic interneurons (C) that send inhibitory projections (gray) to surrounding neurons. This type of circuit creates an "inhibitory surround" (dark gray), so that action potentials generated by neuron **A**, even if rapid and robust, are unable to activate surrounding circuits.

## PATHOPHYSIOLOGY

Because the pathophysiologic mechanisms underlying seizure disorders are only beginning to be determined, seizures are still classified according to their clinical manifestations rather than their biologic underpinnings. Seizures that begin focally (**partial seizures**) are clinically distinct from seizures that begin generally and involve both hemispheres (**generalized seizures**) (Table 13-1). All seizures, however, share the common characteristic of abnormal synchronous discharge. For this to occur, protective mechanisms must be compromised at the cellular and network levels. The direct causes of these changes can be either primary (e.g., such genetic abnormalities as channel defects), secondary (e.g., changes in the neuronal environment induced by toxins or acquired lesions such as strokes or neoplasms), or a combination of the two (e.g., febrile seizures in children). The following examples illustrate the mechanistic links between these initiating factors and subsequent seizure activity.

## PATHOPHYSIOLOGY OF PARTIAL SEIZURES

The partial seizure (Fig. 13-3A) occurs in three specific steps: (1) initiation at the cellular level by an increase in electrical activity, (2) synchronization of surrounding neurons, and (3) spread to adjacent regions of the brain. Seizures are initiated by a sudden depolarization within a group of neurons. This sudden change, called a **paroxysmal depolarizing shift** (PDS), lasts up to 200 ms and results in neuronal generation of an abnormally rapid train of action potentials. Changes in the extracellular milieu, attributable, for instance, to a space-occupying lesion (such as in the introductory case), can have major effects on neuronal burst activity. For example, an increase in extracellular $K^+$ would blunt the effects of $K^+$-mediated after-hyperpolarization by decreasing the magnitude of the $K^+$ gradient between the outside and inside of the cell. Similarly, an increase in excitatory neurotransmitters or modulation of excitatory receptors by other exogenous molecules could increase burst activity. Increased burst activity could also result from properties intrinsic to the cell, such as abnormal channel conductance or altered membrane characteristics.

Because of surround inhibition, local discharges are often contained within a so-called **focus,** and do not induce symptomatic pathology. These local discharges can be seen on an **EEG** as sharp **"interictal"** spikes. Identification of these spikes can be useful in locating the seizure focus in a patient who is not actively undergoing a seizure. There are several pathways, however, whereby the seizure focus can override surround inhibition. Repetitive firing of neurons increases extracellular $K^+$. As described earlier, this weakens $K^+$-mediated hyperpolarization, allowing the seizure activity to spread. Rapidly firing neurons also open depolarization-sensitive NMDA channels (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission) and accumulate $Ca^{2+}$ in their synaptic terminals, both of which increase the likelihood of signal propagation and local synchronization. In many cases, however, it seems that the most important compromise of surround inhibition occurs at the level of GABAergic transmission. Decreases in GABA-mediated

**TABLE 13-1** Classification of Epileptic Seizures

| TYPE | SYMPTOMS/KEY FEATURES |
|---|---|
| **PARTIAL SEIZURES** | |
| Simple partial | ▪ Symptoms vary depending on location of abnormal activity in the brain: involuntary, repetitive movement (motor cortex), paresthesias (sensory cortex), flashing lights (visual cortex), etc.<br>▪ ***Consciousness is preserved***<br>▪ Spread to ipsilateral regions within cortex (e.g., "Jacksonian march") |
| Complex partial (also "Temporal lobe epilepsy") | ▪ Symptoms typically result from abnormal activity in the temporal lobe (amygdala, hippocampus) or frontal lobe<br>▪ ***Altered consciousness*** (cessation of activity, loss of contact with reality)<br>▪ Often associated with involuntary "automatisms" ranging from simple repetitive movements (lip smacking, hand wringing) to highly skilled activity (driving, playing musical instrument)<br>▪ Impaired memory of ictal phase<br>▪ Classically preceded by an aura |
| Partial with secondary generalization: | ▪ Initially manifests with symptoms of simple or complex partial seizure<br>▪ Evolves into a tonic–clonic seizure with sustained contraction (tonic) followed by rhythmic movements (clonic) of all limbs<br>▪ ***Loss of consciousness***<br>▪ Preceded by aura |
| **PRIMARY GENERALIZED SEIZURES** | |
| Absence seizure (petit mal) | ▪ Sudden, brief interruption of consciousness<br>▪ Blank stare<br>▪ Occasional motor symptoms such as lip smacking, rapid blinking<br>▪ Not preceded by an aura |
| Myoclonic seizure | ▪ Brief (1 second or less) muscle contraction. Symptoms may occur in individual muscle or generalize to all muscle groups of the body (the latter can result in falling)<br>▪ Associated with systemic diseases such as uremia, hepatic failure, hereditary degenerative conditions, Creutzfeldt–Jakob disease |
| Tonic–clonic (grand mal) | ▪ Symptoms as described above, but onset is abrupt, and not preceded by symptoms of partial or complex seizure |

inhibition—because of exogenous factors, degeneration of GABAergic neurons, or changes at the receptor level—are major factors that aid in the synchronization of a seizure focus.

If the synchronizing focus is sufficiently strong, the abnormal, synchronized firing from a small neural network will begin to spread to neighboring regions of the cortex. During this spread to neighboring areas, the patient may experience an **aura**—a conscious "warning" of the spread of the seizure. In the introductory case, Rob's aura manifested as a blank, fearful stare. Although the aura is usually stereotypical for a given patient, a wide variety of auras exist. These include a sense of fear and confusion, disturbances of memory (e.g., déjà vu) or language, altered sensations, or an olfactory hallucination. As the seizure continues to spread, it can lead to additional clinical manifestations; the specific manifestation depends on the brain regions that become involved. In the introductory case, the clinical symptoms initially started with shaking of the hands and progressed to the arm and then to the leg. This is a **Jacksonian march** (named after the English neurologist Hughlings Jackson, who first described the symptoms), where the clinical symptoms result from spread of synchronous activity across the motor homunculus.

## PATHOPHYSIOLOGY OF SECONDARY GENERALIZED SEIZURES

Partial seizures may become generalized by spreading along diffuse connections to involve both cerebral hemispheres. This is known as a **"secondarily generalized seizure"** (Fig. 13-3B). Typically, seizures spread to distant sites by following normal circuits, and this spread can occur through several pathways. **U fibers** connect various regions of the cortex; the **corpus callosum** allows for spread between hemispheres; and **thalamocortical projections** provide a pathway for diffuse synchronized spread throughout the brain. Once seizure activity spreads to involve both hemispheres, a patient usually loses consciousness.

Among the secondarily generalized seizures, the **tonic–clonic** subtype is the most common. In the introductory

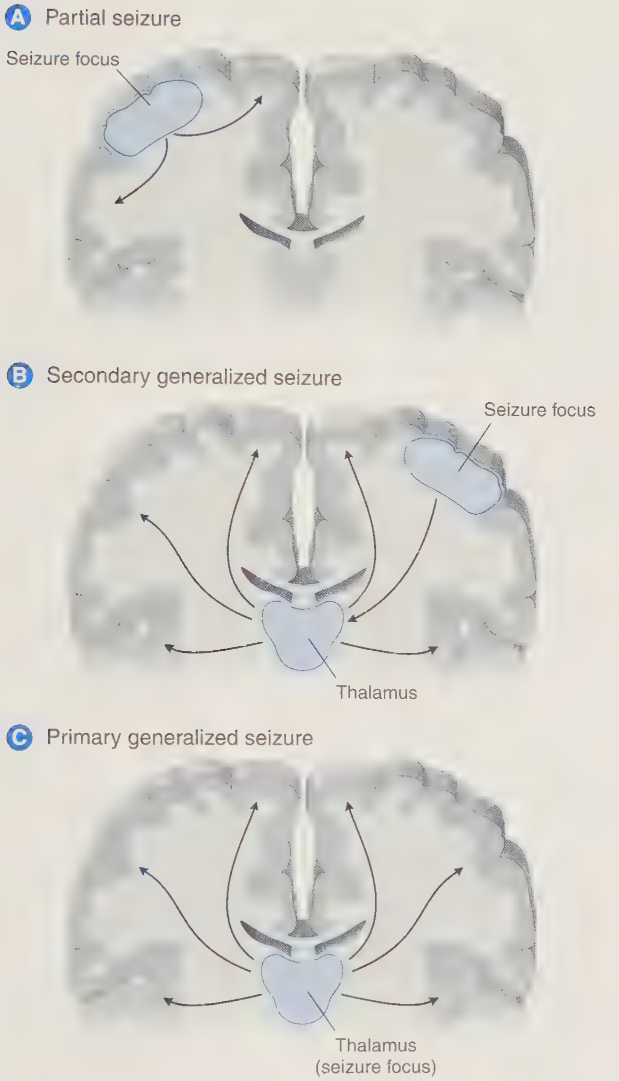


**Figure 13-3. Pathways of Seizure Propagation.** **A.** In a partial seizure, paroxysmal activity begins in a seizure focus (blue) and spreads to adjacent areas via diffuse neuronal connections. When activity is confined to one region of the cortex that serves a basic function, such as motor movement or sensation, and there is no change in the patient's mental status, the seizure is referred to as a simple partial seizure. Seizures that involve brain regions serving more complex functions such as language, memory, and emotions are referred to as complex partial seizures. **B.** In a secondary generalized seizure, paroxysmal activity begins in a focus but then spreads to subcortical areas. Diffuse connections from the thalamus then synchronize the spread of activity to both hemispheres. **C.** Primary generalized seizures, such as the absence seizure, result from abnormal synchronization between thalamic and cortical cells (see Fig. 13-5B).

clinical case, Rob underwent a period where he appeared to be contracting every muscle in his body, followed by an episode of uncontrolled shaking of all four limbs. These clinical symptoms can be understood at the level of abnormal channel activity (Fig. 13-4). The initial phase of the tonic–clonic seizure is associated with a sudden loss of GABA input, which leads to a long train of firing lasting for several seconds. This sustained, rapid firing manifests clinically as contraction of both agonist and antagonist muscles, and is referred to as the **tonic** phase. Eventually, as GABA-mediated inhibition begins to be restored, AMPA-mediated and NMDA-mediated excitation starts to oscillate with the inhibitory component. This oscillatory pattern (when involving the motor cortex) results in **clonic** or shaking movements of the body. With time, the GABA-mediated inhibition prevails, and the patient becomes flaccid and remains unconscious during the **postictal** period until normal brain function returns.

## PATHOPHYSIOLOGY OF PRIMARY GENERALIZED SEIZURES

Primary generalized seizures differ from partial seizures in both pathophysiology and etiology (Fig. 13-3C). In contrast to the partial seizure, where synchronicity begins with sudden trains of action potentials within an aggregate of neurons and subsequently spreads to adjacent regions, the primary generalized seizure emanates from central brain regions and then spreads rapidly to both hemispheres. These seizures do not necessarily begin with an aura (which is an important method of clinically distinguishing primary generalized seizures from partial seizures that secondarily generalize).

Currently, the best understood of the primary generalized seizures is the **absence seizure** (also known as the **petit mal seizure**). Absence seizures are characterized by sudden interruptions in consciousness that are often accompanied by a blank stare and occasional motor symptoms, such as rapid blinking and lip smacking. Absence seizures are thought to result from abnormal synchronization of thalamocortical and cortical cells. The underlying pathophysiology of absence seizures is based on the observation that patients experiencing absence seizures have EEG readings somewhat similar to the patterns generated during **slow-wave (stage 3) sleep.**

Relay neurons connecting the thalamus to the cortex exist in two different states depending on the level of wakefulness (Fig. 13-5A). During the awake state, these neurons function in **transmission mode,** whereby incoming sensory signals are faithfully transmitted to the cortex. During sleep, however, the transient, bursting activity of a unique, dendritic **T-type calcium channel** alters incoming signals so that output signals to the cortex have an oscillatory firing rate, which, on an EEG, has a characteristic "spike and wave" readout. In this slow-wave sleep state, sensory information is not transmitted to the cortex.

For reasons not yet understood, absence seizures are associated with activation of the T-type calcium channel during the awake state (Fig. 13-5B). Because this channel is active only when the cell is hyperpolarized, a number of potential factors could act to activate the channel during the awake state. These factors include an increase in intracellular $K^+$, increased GABAergic input from the reticular nucleus, or a loss of excitatory input. A variety of studies have shown that the activity of the T-type calcium channel in the relay neurons is essential to the 3-per-second spike and wave activity observed in absence seizures. Because of its important pathophysiologic role, the T-type calcium channel is a primary target in the pharmacologic treatment of absence seizures.

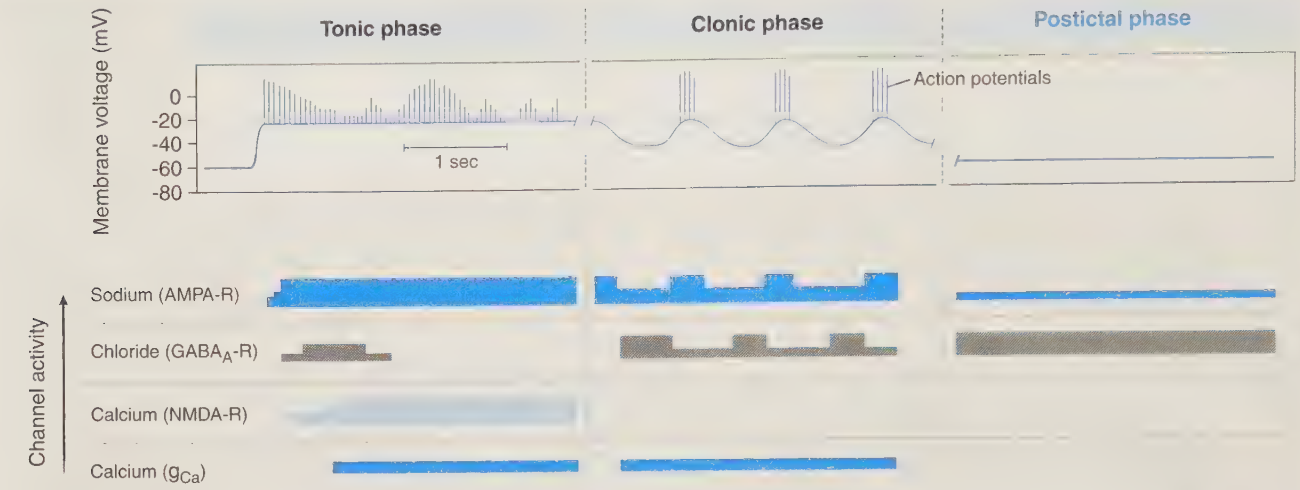


Figure 13-4. Abnormal Channel Activity in the Tonic–Clonic Seizure. The tonic phase of the tonic–clonic seizure is initiated by a sudden loss of GABA-mediated surround inhibition. Loss of inhibition results in a rapid train of action potentials, which manifests clinically as tonic contraction of the muscles. As GABAergic innervation is restored, it begins to oscillate rhythmically with the excitatory component. The oscillation of excitatory and inhibitory components manifests clinically as clonic movements. The postictal phase is characterized by enhanced GABA-mediated inhibition.

## PHARMACOLOGIC CLASSES AND AGENTS

The current approach to treating a patient with epilepsy depends in part on the type of seizure(s) experienced by the patient. Patients with partial seizures, with or without secondary generalization, are typically treated pharmacologically with antiepileptic drugs. In such patients, there is also an attempt to determine whether the seizures are caused by an identifiable focal lesion that can be removed surgically or ablated by other means. Antiepileptic drugs (AEDs) remain the mainstay of treatment for patients with generalized seizures as well.

Mechanistically, the efficacy of AEDs centers on manipulation of ion channel activity. As discussed above, physiologic protection against repetitive firing occurs via inhibition at two levels: the cellular level ($Na^+$ channel inactivation), and the network level (GABA-mediated inhibition). Accordingly, currently available AEDs fall into three main categories: 1) drugs that enhance $Na^+$ channel-mediated inhibition, 2) drugs that inhibit the T-type calcium channel, and 3) drugs that enhance GABA-mediated inhibition.

Although AEDs fall into several different mechanistic classes, it is important to keep in mind that the therapeutic efficacy of many of the AEDs is only partially explained by the known mechanisms described below, primarily because the AEDs act pleiotropically. Valproic acid, for example, does stabilize $Na^+$ channels, but at an appropriate concentration, the drug may have effects on GABA metabolism as well. Thus, although initial in vitro studies may suggest that a drug is best suited for the treatment of one particular type of seizure, other seizure types may also respond to the drug. (One clinical benefit of this pleiotropy is that many of the drugs are interchangeable to the extent that minimization of side effects is often the main criterion underlying the choice of AED.) The classification below is shown only for simplicity and is based upon the primary target of the drug.

## DRUGS THAT ENHANCE NA$^+$ CHANNEL-MEDIATED INHIBITION

Each individual neuron in the brain is equipped with the machinery to prevent rapid, repetitive firing. As discussed above, depolarization of the neuronal membrane results in sodium channel inactivation. This inactivation of the $Na^+$ channel provides a key checkpoint in the prevention of repetitive firing within a potential seizure focus. Although changes in the extracellular milieu, such as altered ion concentration, can override this checkpoint, the AEDs **phenytoin, carbamazepine, lamotrigine,** and **valproate** enhance inhibition at the single cell level by acting directly on the $Na^+$ channel (Fig. 13-6A).

In general, *antiepileptic drugs that act on $Na^+$ channels show strong specificity for the treatment of partial and secondary generalized seizures.* This is consistent with their molecular profile. The $Na^+$ channel blockers act in a use-dependent manner, much like the action of lidocaine on peripheral nerves (see Chapter 9, Local Anesthetic Pharmacology). Thus, neurons that fire rapidly are particularly susceptible to inhibition by this class of drug. Conversely, many $Na^+$ channel blockers (particularly those that act only at the $Na^+$ channel, such as phenytoin) have little effect on absence seizures. Presumably, the rate of cyclical $Na^+$ channel opening and closing within the thalamocortical cells that are activated during absence seizures is too slow to be amenable to inhibition through use-dependent $Na^+$ channel inactivation.

### Phenytoin

Phenytoin acts directly on the $Na^+$ channel to slow the rate of channel recovery from inactivation. As discussed above and in Chapter 9, the $Na^+$ channel exists in three conformations—open, closed, and inactive—the probability of a channel existing in each state depends on the transmembrane voltage difference (Fig. 13-1). By slowing the rate of recovery from

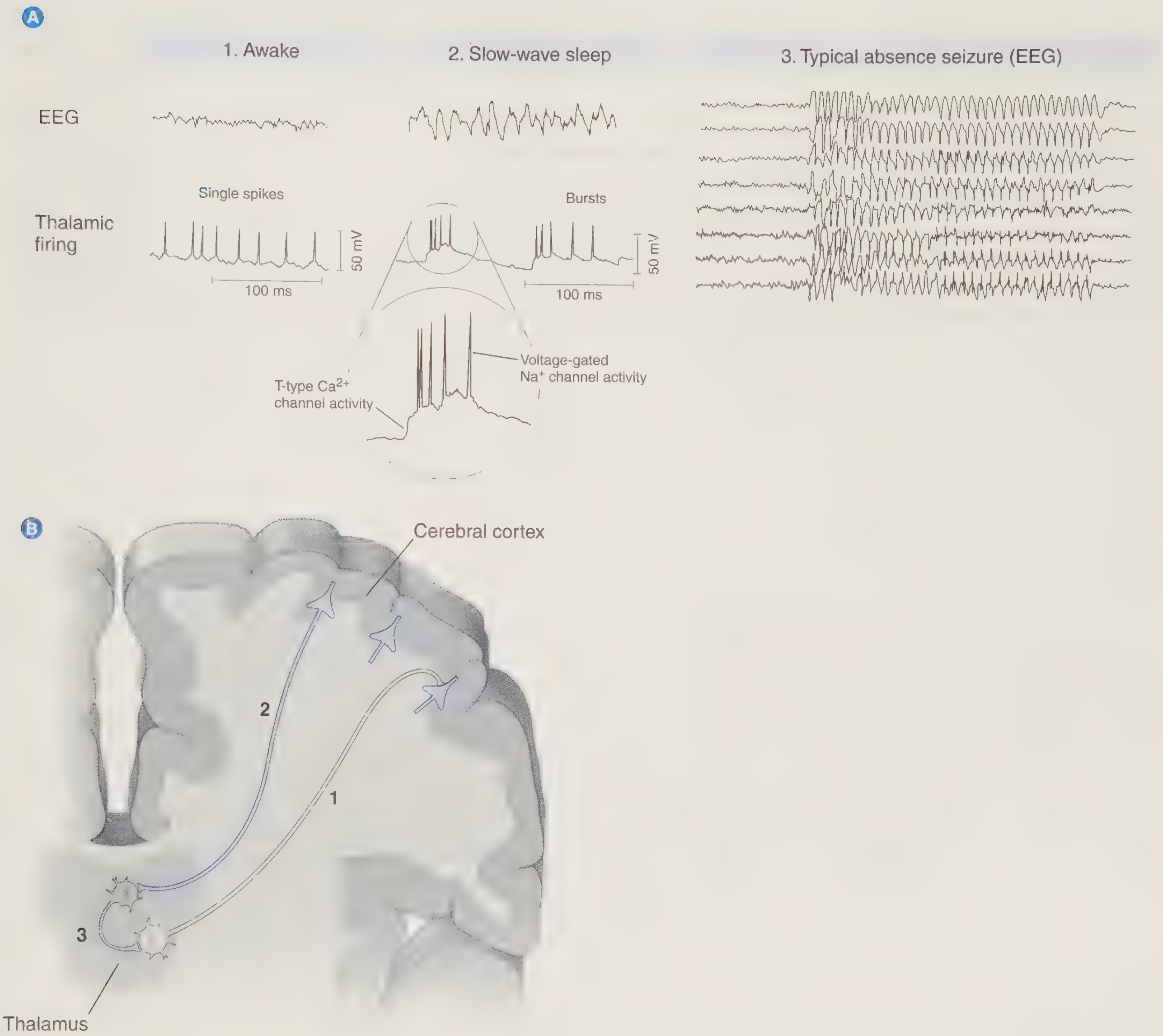


**Figure 13-5. Mechanism of Absence Seizure.** **A.** EEG recordings of patients experiencing absence seizures are similar to "sleep spindle" patterns generated during slow-wave sleep. The 3 per second oscillatory pattern is generated by the burst activity of a dendritic T-type calcium channel in the thalamus. 1. During the awake state, relay neurons of the thalamus are in "transmission mode," in which incoming signals are faithfully transmitted to the cortex as single spikes. These signals to the cortex register on the EEG as small, desynchronized, low voltage waves. 2. During slow-wave sleep, signals relayed through the thalamus are altered because of the bursting activity of a dendritic T-type calcium channel (see below). During this stage, called "burst mode," sensory information is not transmitted to the cortex. 3. Absence seizures result from abnormal activation of the T-type calcium channel during the awake state, resulting in a similar spike-and-wave EEG pattern. **B.** The absence seizure is generated by a self-sustaining cycle of activity between the thalamus and the cortex. Synchronicity is initiated by hyperpolarization of the thalamic relay neurons (light gray). This occurs normally during slow-wave sleep caused by GABAergic input from the reticular thalamic nucleus (dark gray). The factors that cause hyperpolarization in relay neurons during an absence seizure are poorly understood. 1. Hyperpolarization of relay neurons induces burst activity of the T-type calcium channel, resulting in synchronous depolarization in the cortex via excitatory connections. This large depolarization in the cortex registers as a spike-and-wave pattern on the EEG. 2. Excitatory input from the cortex activates the reticular thalamic neurons. 3. The activated GABAergic reticular neurons hyperpolarize the thalamic relay neurons and reinitiate the cycle.

the inactive state, phenytoin increases the threshold for action potentials and prevents repetitive firing. This has the effect of stabilizing the seizure focus by preventing the paroxysmal depolarizing shift (PDS) that initiates the partial seizure. In addition, phenytoin prevents the rapid spread of seizure activity to other neurons, accounting for its efficacy in secondarily generalized seizures.

Importantly, phenytoin targets $Na^+$ channels in a use-dependent manner. Thus, only channels that are opened and closed at high frequency (i.e., those involved in the PDS) are likely to be inhibited. This use-dependency lessens the effects of phenytoin on spontaneous neuronal activity and avoids many of the side effects noted with GABA agonists (which are not use-dependent).

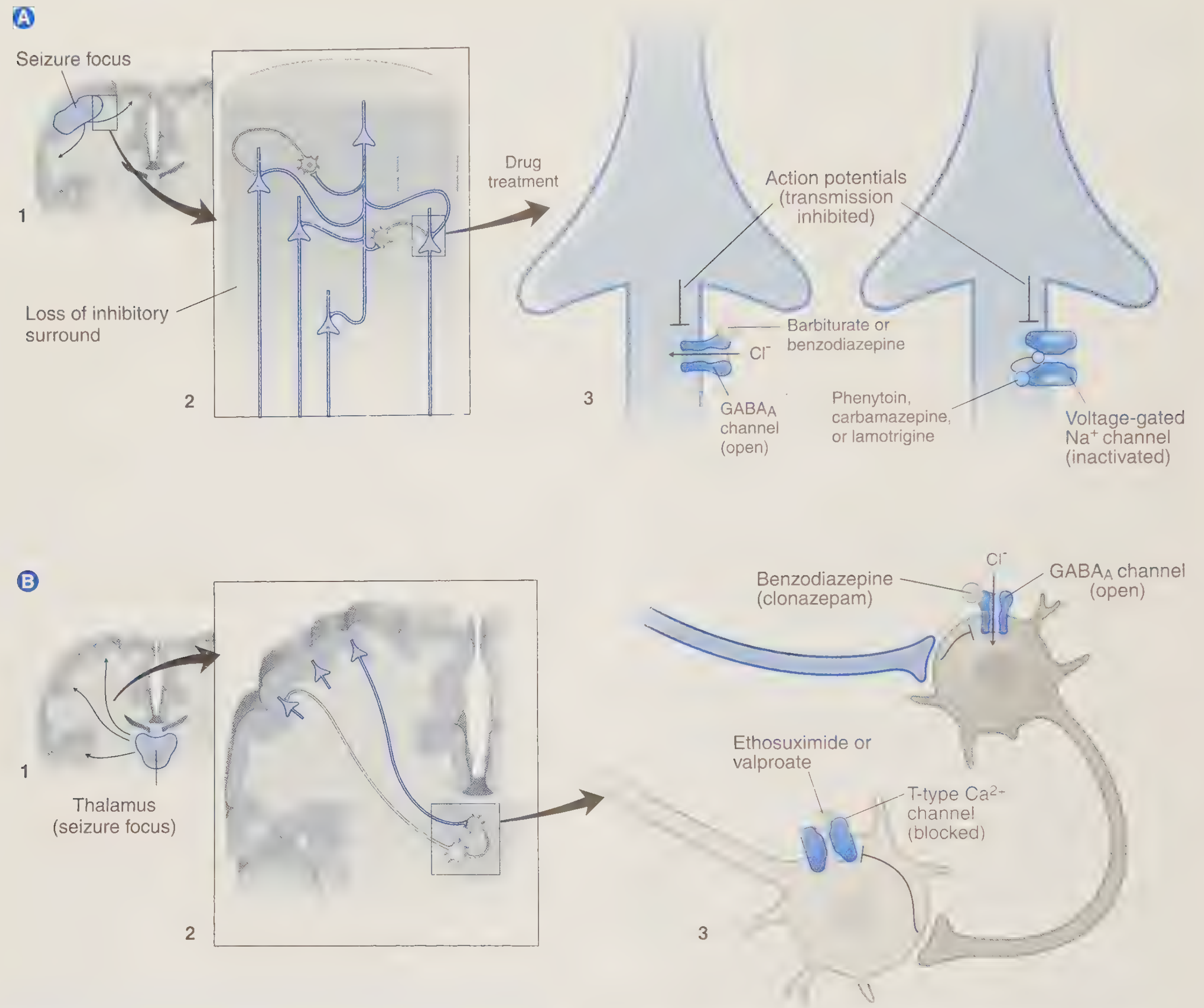

A
Seizure focus
1
Loss of inhibitory surround
2
Drug treatment
3
Action potentials (transmission inhibited)
Barbiturate or benzodiazepine
Cl⁻
GABAA channel (open)
Phenytoin, carbamazepine, or lamotrigine
Voltage-gated Na⁺ channel (inactivated)
B
1
Thalamus (seizure focus)
2
3
Benzodiazepine (clonazepam)
Cl⁻
GABAA channel (open)
Ethosuximide or valproate
T-type Ca2+ channel (blocked)

**Figure 13-6. Mechanisms of Pharmacotherapy for Seizures.** **A.** The partial seizure (1) results from rapid, uncontrolled neuronal firing and a loss of surround cell inhibition (2). Antiepileptic drugs act at two molecular targets to enhance inhibition and prevent spread of synchronous activity (3): Barbiturates and benzodiazepines prevent seizure spread by acting on the $GABA_A$ receptor to potentiate GABA-mediated inhibition. $Na^+$ channel inhibitors such as phenytoin, carbamazepine, and lamotrigine prevent rapid neuronal firing by selectively prolonging $Na^+$ channel inactivation in rapidly firing neurons (see Figs. 9-8, 9-9). **B.** The absence seizure (1) is caused by a self-sustaining cycle of activity generated between thalamic and cortical cells (2). Antiepileptic drugs prevent this synchronous thalamocortical cycle (3) by acting at two molecular targets: clonazepam, a benzodiazepine, potentiates $GABA_A$ channels in the reticular thalamic nucleus, thus decreasing the activation of the inhibitory reticular neurons and decreasing the hyperpolarization of the thalamic relay neurons. T-type calcium channel inhibitors such as ethosuximide and valproate prevent the burst activity of thalamic relay neurons that is required for synchronous activation of cortical cells.

Because of its use-dependent blockade, as well as its ability to prevent sudden rapid firing, phenytoin is a major drug of choice for partial seizures and tonic clonic seizures. It is not used in absence seizures. The pharmacokinetics and drug interactions of phenytoin are complex and play a decisive role in the choice between phenytoin and similarly acting drugs such as carbamazepine.

Phenytoin is more than 95% bound to plasma albumin. Phenytoin is inactivated by metabolism in the liver and at typical doses has a plasma half-life of about 24 hours. Phenytoin metabolism shows properties of saturation kinetics, whereby small increases in dosage can cause large and often unpredictable increases in the plasma concentration (see Chapter 3, Pharmacokinetics). These increases in plasma phenytoin concentration increase the risk of side effects, including ataxia, nystagmus, incoordination, confusion, gum hyperplasia, megaloblastic anemia, hirsutism, facial coarsening, and a systemic skin rash.

Phenytoin inactivation by the hepatic microsomal P450 enzyme system is susceptible to alteration by several drugs. Drugs that inhibit the P450 system, such as chloramphenicol, dicumarol, cimetidine, disulfiram, and isoniazide, increase phenytoin plasma concentration. Carbamazepine, an antiepileptic drug (see below) that induces the liver P450 system, increases the metabolism of phenytoin; thereby, lowering phenytoin plasma concentrations when these drugs are used together. Similarly, phenytoin, because of its ability to induce the hepatic P450 system, increases the metabolism of drugs that are inactivated by this system. Some of these drugs include oral contraceptives, quinidine, doxycycline, cyclosporine, methadone, and levodopa.

### Carbamazepine

Although structurally unrelated to phenytoin, carbamazepine seems to exert its antiseizure activity in a manner similar to phenytoin. That is, carbamazepine slows the rate of recovery of $Na^+$ channels from the inactive state. This has the effect of suppressing seizure foci (by preventing the PDS) as well as preventing rapid spread of activity from the seizure focus. The metabolite of carbamazepine, 10,11-epoxycarbamazepine, also acts to slow $Na^+$ channel recovery, and may be responsible for some of the therapeutic effects of this drug.

Carbamazepine is often the drug of choice for partial seizures (simple and complex) because of its dual action in the suppression of seizure foci and the prevention of spread of activity. The half-life of carbamazepine is initially between 10 and 20 hours and is further reduced with chronic treatment (because of P450 induction), requiring patients to take several doses daily. Metabolism of carbamazepine is linear (i.e., it exhibits first-order kinetics); this property makes carbamazepine a more attractive choice than phenytoin for patients with other complications or potential drug interactions.

### Lamotrigine

As with phenytoin and carbamazepine, lamotrigine stabilizes the neuronal membrane by slowing $Na^+$ channel recovery from inactivation. Lamotrigine may also have other, undetermined, mechanisms of action; this hypothesis is based on the clinical observation that lamotrigine has wider clinical applications than otherwise similar $Na^+$ channel inhibitors.

Lamotrigine is a useful alternative to phenytoin and carbamazepine as a treatment for partial and tonic–clonic seizures. Surprisingly, and for reasons not correlated with its defined mechanism, lamotrigine has been shown to be effective in the treatment of atypical absence seizures. It is the third drug of choice for treatment of absence seizures, after ethosuximide and valproate (see below).

## DRUGS THAT ACT AT THE T-TYPE CALCIUM CHANNEL

*Drugs that fall into this category are effective in the treatment of absence (petit mal) seizures.* As discussed previously, the T-type calcium channel is depolarized and inactive during the awake state (Fig. 13-5B). In absence seizures, paroxysmal hyperpolarization is thought to activate the channel during the awake state, initiating the spike and wave discharges characteristic of this seizure type.

### Ethosuximide

In vitro, *ethosuximide* has a highly specific molecular profile. In experiments on thalamocortical preparations from rats and hamsters, ethosuximide has been shown to reduce low threshold T-type currents in a voltage-dependent manner. This inhibition occurs without altering the voltage dependence or recovery kinetics of the $Na^+$ channel. Ethosuximide does not have any effect on GABA-mediated inhibition.

Ethosuximide is often the first line therapy for uncomplicated absence seizures. Consistent with its molecular profile as a specific T type $Ca^{2+}$ channel antagonist, ethosuximide is not effective in the treatment of partial or secondary generalized seizures.

### Valproic Acid

As is the case for many other AEDs, **valproate** acts pleiotropically in vitro. As with phenytoin and carbamazepine, valproate slows the rate of $Na^+$ channel recovery from inactivation. At slightly higher concentrations than those necessary to limit repetitive firing, valproate has also been shown to limit the activity of the low threshold T-type calcium channel.

Another proposed mechanism of valproate activity occurs at the level of GABA metabolism. In vitro, valproate increases the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis, while it inhibits the activity of enzymes that degrade GABA. Taken together, these effects are thought to increase the availability of GABA in the synapse.

Perhaps because of its many potential sites of action, valproate is one of the most effective antiepileptic drugs for the treatment of patients with generalized epilepsy syndromes having mixed seizure types. Valproate is also the drug of choice for patients with idiopathic generalized seizures and is used for the treatment of absence seizures that do not respond to ethosuximide. Valproate is also commonly used as an alternative to phenytoin and carbamazepine for the treatment of partial seizures.

## DRUGS THAT ENHANCE GABA-MEDIATED INHIBITION

In contrast to $Na^+$ channel blockers and T-type $Ca^{2+}$ channel inhibitors, whose mechanistic properties correlate well with their clinical activity, the GABA-mediated inhibitors have more varied effects, and tend not to be as interchangeable. This is largely because of the diversity of GABA receptors in the brain. The $GABA_A$ receptor channel has five subunits, with at least two alternative splice variants of several of the subunits (see Chapter 10). There are at least 10 known subtypes of the $GABA_A$ receptor, with varying distributions of these subtypes throughout the brain. Although barbiturates and benzodiazepines both increase $Cl^-$ influx through $GABA_A$ channels, benzodiazepines act on a specific subset of $GABA_A$ channels whereas barbiturates appear to act on all $GABA_A$ channels. This difference in specificity results in distinct clinical profiles. Drugs that nonspecifically increase GABA content (e.g., through enhancement of synthetic pathways) tend to have a profile similar to the barbiturates.

### Gabapentin

Several potential mechanisms have been described for this drug, although none have been substantiated. In vitro, **gabapentin** increases the content of GABA in neurons and in glial cells. Similarly, in vivo studies have shown that gabapentin acts to increase GABA content in the brain.

Other proposed mechanisms for gabapentin include inhibitory action at the $Na^+$ channel, as well as inhibitory action at the voltage-sensitive $Ca^{2+}$ channel. The latter effect could potentially modify neurotransmitter release.

Gabapentin is used as second-line therapy in the treatment of partial seizures. Although not generally as effective as other AEDs, gabapentin has the unique advantage of having no known drug interactions.

### Benzodiazepines (Diazepam, Clonazepam)

Benzodiazepines increase the affinity of GABA for its receptor and, thereby, increase $Cl^-$ current through the channel (see Chapter 10). This action has the dual effect of suppressing the seizure focus (by raising the threshold of the action potential) and strengthening surround inhibition. Thus, such benzodiazepines as **diazepam** and **midazolam** are particularly well suited for the treatment of partial and tonic–clonic seizures. The benzodiazepines cause prominent side effects, however, including dizziness, ataxia, and drowsiness. Thus, these drugs are usually used only to ablate seizures acutely.

Of the benzodiazepines, **clonazepam** is unique because it can also inhibit T-type $Ca^{2+}$ channel currents in in vitro preparations of thalamocortical circuits. Clonazepam acts specifically at GABA receptors in the reticular nucleus (Fig. 13-5B). By augmenting inhibition in these neurons and essentially "turning off" the nucleus, clonazepam prevents GABA-mediated hyperpolarization to the thalamus. This indirectly inactivates the T type $Ca^{2+}$ channel, which is the channel thought to be responsible for generation of the absence seizure (see above). However, as with diazepam, clonazepam is limited in use because of its extensive side effects. Clonazepam is the fourth drug of choice in the treatment of absence seizures, after lamotrigine.

### Barbiturates (Phenobarbital)

**Phenobarbital** binds to an allosteric site on the GABA receptor and, thereby, potentiates the action of endogenous GABA, increasing the duration of $Cl^-$ influx (see Chapter 10). Barbiturates also display weak agonist activity at the GABA channel, perhaps furthering the ability of this drug to increase $Cl^-$ influx. This enhancement of GABA-mediated inhibition, similar to that of the benzodiazepines, may explain the effectiveness of phenobarbital in the treatment of partial seizures and tonic–clonic seizures.

In contrast to the benzodiazepines, which are sometimes useful in treating the spike-wave discharges of the absence seizure, the barbiturates may actually exacerbate this type of seizure. This exacerbation may be caused by two important factors. First, barbiturates act at all GABA receptors. Although benzodiazepines selectively augment GABA inhibition in the reticular nucleus, barbiturates potentiate GABA receptors in both the reticular nucleus and the thalamic relay cells. Importantly, the latter effect enhances the T-type calcium currents that are responsible for the absence seizure (Fig. 13-5B). Second, unlike benzodiazepines, which are purely allosteric enhancers of endogenous GABA activity, barbiturates can also act on the GABA channel in the absence of the native ligand. The latter property may function to increase nonspecific activity of the barbiturates.

Phenobarbital is used primarily as an alternative drug in the treatment of partial seizures and tonic–clonic seizures. Because of the pronounced sedative side effects of this drug, however, its clinical use has been decreasing as more effective antiepileptic medications have become available.

## *Conclusion and Future Directions*

In recent years, improved understanding of the physiology and pathophysiology of neuronal signaling in the CNS has led to a more thorough understanding of the current antiepileptic drugs, as well as the design and discovery of novel agents. Under physiologic conditions, $Na^+$ channel inactivation and GABA-mediated surround inhibition prevent uncontrolled, rapid spread of electrical activity. There are, however, numerous potential alterations in the brain that can weaken these inhibitory forces, such as damage and degeneration of GABAergic neurons, abnormal ion gradients induced by space-occupying lesions, and gene mutations that alter channel function.

The AEDs described in this chapter restore the inherent inhibitory capacity of the brain. These include such drugs as phenytoin, which increases $Na^+$ channel inactivation, as well as such drugs as clonazepam, which enhances GABA-mediated inhibition. Newer classes of AEDs that have recently reached the clinics extend this repertoire by acting not only to decrease $Na^+$-dependent burst firing and GABA-mediated inhibition, but also to manipulate other channels and receptors involved in the regulation of signal propagation. These new molecular targets include modulation of such excitatory receptors as the NMDA receptor and modulation of the $Ca^{2+}$ channel required for neurotransmitter release.

Despite increased understanding of the mechanisms of certain seizure types, the efficacy of many of the anticonvulsants is only partially explained by their known molecular profile. Hence, current decisions about therapy are often driven more by empirical example than by known molecular mechanisms. As knowledge of the mechanisms of various seizure types and antiseizure drugs increases, the application of a more rational, mechanism-based pharmacology will become increasingly possible.

## *Suggested Readings*

Lowenstein DH. Seizures and epilepsy. In: Harrison's Principles of Internal Medicine. 15th Ed. New York: McGraw Hill, 2000. (*Discussion of seizure pathophysiology. Extensive discussion of clinical use of antiepileptic drugs.*)

Westbrook GL. Seizures and epilepsy. In: Kandel ER, Schwartz JH, Jessell TM (eds.). Principles of Neuroscience. New York: McGraw-Hill, 2000. (*Detailed description of normal electrical signaling and seizure pathophysiology.*)

## Drug Summary Table Pharmacology of Abnormal Electrical Neurotransmission

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **Na⁺ CHANNEL INHIBITORS**<br>*Mechanism—Enhance Na⁺ channel-mediated inhibition by slowing Na⁺ channel recovery from the inactivated state* | | | | |
| **Phenytoin** | Partial, tonic–clonic seizures | CNS: Ataxia, nystagmus, incoordination, confusion<br>Systemic: Gum hyperplasia, megaloblastic anemia, hirsutism, facial coarsening, skin rash | Levels increased by chloramphenicol, dicumarol, cimetidine, disulfiram, isoniazid<br>Levels decreased by carbamazepine and phenobarbital<br>Increases metabolism of anticoagulants, quinidine, doxycycline, cyclosporine, methadone, levodopa, oral contraceptives, antiepileptics | More than 95% bound to plasma albumin<br>At low doses, half-life is 24 h. At higher doses, phenytoin saturates the P450 system, such that small changes in dosage can result in large changes in plasma concentration, thereby increasing the risk of side effects |
| **Carbamazepine** | Partial, tonic–clonic seizures | CNS: Ataxia, vertigo, dizziness, diplopia<br>Systemic: Aplastic anemia, hepatotoxicity, gastrointestinal irritation, hyponatremia | Levels increased by erythromycin, isoniazid, cimetidine, diltiazem, propoxyphene<br>Levels decreased by phenobarbital, phenytoin | Metabolism follows linear (first-order) kinetics |
| **Lamotrigine** | Partial, tonic–clonic, atypical absence seizures | CNS: Dizziness, diplopia, sedation, ataxia, headache<br>Systemic: Skin rash, Stevens–Johnson syndrome | Levels increased by valproic acid.<br>Levels decreased by carbamazepine, phenobarbital, phenytoin | |
| **T-TYPE CALCIUM CHANNEL INHIBITORS**<br>*Mechanism—Reduce activity of low threshold T-type calcium channel* | | | | |
| **Ethosuximide** | Absence seizures | CNS: Ataxia, lethargy, headache<br>Systemic: GI irritation, skin rash, bone marrow suppression | | |
| **Valproic acid** | Tonic–clonic, absence, atypical absence, partial seizures | CNS: Ataxia, sedation, tremor<br>Systemic: Hepatotoxicity, thrombocytopenia, GI irritation, weight gain, hyperammonemia | Levels decreased by carbamazepine, phenobarbital, phenytoin | |

*(Continued)*

## Drug Summary Table Pharmacology of Abnormal Electrical Neurotransmission *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **GABA CHANNEL POTENTIATORS**<br>*Mechanism—Potentiate GABA-mediated inhibition* | | | | |
| **Gabapentin** | Partial seizures | CNS: Sedation, dizziness, ataxia, fatigue<br>Systemic: GI irritation | No known significant interactions | |
| **Benzodiazepines** | Partial, absence seizures | CNS: Dizziness, ataxia, drowsiness<br>Systemic: Anorexia | Levels decreased by carbamazepine, phenobarbital | |
| **Barbiturates** | Partial, tonic–clonic seizures | CNS: Sedation, ataxia, confusion, dizziness, decreased libido, depression<br>Systemic: Skin rash | Levels increased by valproic acid, phenytoin | |
| **NEW ANTIEPILEPTIC DRUGS**<br>*Mechanisms under investigation* | | | | |
| **Tiagabine** | Partial seizures (adjunct therapy) | CNS: Confusion, sedation, dizziness, depression, psychosis<br>Systemic: GI irritation | Levels decreased by carbamazepine, phenytoin, phenobarbital | |
| **Topiramate** | Partial, tonic–clonic seizures (adjunct therapy) | CNS: Sedation, psychomotor slowing, fatigue, speech or language problems<br>Systemic: Renal stones | Levels decreased by phenytoin, carbamazepine | |
| **Levetiracetam** | Partial seizures (adjunct therapy) | CNS: Sedation, fatigue, incoordinaion, psychosis<br>Systemic: Anemia, leukocytopenia | None known | |
| **Zonisamide** | Partial, tonic–clonic seizures (adjunct therapy) | CNS: Sedation, dizziness, confusion, headache<br>Systemic: Anorexia, renal stones | Levels decreased by carbamazepine, phenytoin, phenobarbital | |

In this table, "partial seizures" includes simple partial, complex partial, and secondarily generalized seizures.

# 14

# General Anesthetic Pharmacology

Jacob Wouden and Keith W. Miller

**Introduction**
**Case**
**Pharmacodynamics of Inhaled Anesthetics**
  The Minimum Alveolar Concentration (MAC)
  Therapeutic and Analgesic Indices
  The Meyer–Overton Rule
**Pharmacokinetics of Inhaled Anesthetics**
  Concepts from Respiratory Physiology
    *Local Equilibration*
    *Global Equilibration*
  The Uptake Model
    *Equilibration of Alveolar with Inspired Partial Pressure*
    *Equilibration of Tissue with Alveolar Partial Pressure*
    *The Rate-Limiting Step*
  Applications of the Uptake Model
    *Effects of Changes in Ventilation*
    *Effects of Changes in Cardiac Output*
    *Effects of Age*
    *Effects of Abnormal States*
    *Control of Induction*
  Recovery
**Pharmacology of General Anesthetics and Adjuvants**
  Inhaled Anesthetic Agents
  Intravenous Anesthetic Agents
  Adjuvant Drugs
  Balanced Anesthesia
**Mechanism of Action of General Anesthetics**
  The Meyer–Overton Rule and the Lipid Solubility Hypothesis
  Effects on Ion Channels
**Conclusion and Future Directions**
**Suggested Readings**
**Appendix A: Abbreviations and Symbols**
**Appendix B: Equations**

## INTRODUCTION

Before the discovery of **general anesthetics,** pain and shock severely limited the possibilities for surgical intervention. Postoperative mortality dropped dramatically following the first public demonstration of **diethyl ether** at Massachusetts General Hospital in 1846. Since then, the administration of agents for the induction and maintenance of anesthesia has become a separate medical specialty. The modern anesthesiologist is responsible for all aspects of patient health during surgery. As part of this process, the anesthesiologist controls the depth of anesthesia and maintains homeostatic equilibrium with an arsenal of inhaled and intravenous anesthetics as well as many adjuvant drugs.

General anesthetics induce a generalized, reversible depression of the central nervous system (CNS). Under general anesthesia, there is a lack of perception of all sensations. The anesthetic state includes loss of consciousness, amnesia, and immobility (a lack of response to noxious stimuli), but not necessarily complete analgesia. Other desirable effects provided by anesthetics or adjuvants during surgery may include muscle relaxation, loss of autonomic reflexes, analgesia, and anxiolysis. All of these effects facilitate safe and painless completion of the procedure; some effects are more important in certain types of surgery than others. For example, abdominal surgery necessitates near complete relaxation of the abdominal muscles; whereas, neurosurgery often requires light anesthesia that may be lifted rapidly when the neurosurgeon needs to judge the patient's ability to respond to commands.

This chapter provides a framework for understanding the pharmacodynamics and pharmacokinetics of general anesthetics in the context of physiologic and pathophysiologic variables. After discussing the pharmacology of specific agents and how a balanced anesthetic approach is achieved, the chapter considers what is currently known about the mechanism of action of general anesthetics.

## ■ Case

Matthew is a 7-year-old, 20-kg boy who has been undergoing multidrug chemotherapy for aggressive osteosarcoma of his right femur. The time has now come for a surgical resection.

- 8:00 PM (night before the operation): Dr. Snow, the anesthesiologist, provides reassurance and revisits the importance of fasting after midnight to prevent aspiration of gastric contents while under general anesthesia.

- 6:30 AM: Matthew clings to his mother and appears anxious, cachectic, and in some pain. His vital signs are stable with an elevated pulse of 120 and a blood pressure of 110/75. An oral dose of midazolam (a benzodiazepine; see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission) is given to relieve anxiety and to allow Matthew to separate from his parents.
- 7:00 AM: Dr. Snow injects a small amount of lidocaine subcutaneously (a local anesthetic; see Chapter 9, Local Anesthetic Pharmacology) before inserting an intravenous catheter (which he carefully conceals from Matthew until the last possible moment). Through the catheter, Dr. Snow delivers an infusion of morphine sulfate (an opioid; see Chapter 15 Pharmacology of Analgesia) for analgesia.
- 7:30 AM: Dr. Snow rapidly induces anesthesia with an intravenous bolus of 60 mg (3 mg/kg) of thiopental (a barbiturate; see Chapter 10). Within 45 seconds, Matthew is in a deep anesthetic state. The doctor adds a dose of intravenous succinylcholine (a depolarizing muscle relaxant; see Chapter 7, Cholinergic Pharmacology) to facilitate endotracheal intubation, and Matthew is placed on artificial respiration.
- 7:32 AM: A mixture of inhaled general anesthetics consisting of 2% isoflurane, 50% nitrous oxide, and 48% oxygen is provided through the ventilator to maintain the anesthetic state.
- 7:50 AM: Matthew shows no response, either through movement or increased sympathetic tone (e.g., increased heart rate, increased blood pressure), to the first surgical incision.
- 8:20 AM: Dr. Snow notices with a start that Matthew's pulse has fallen to 55 and his blood pressure to 85/45. Berating himself for forgetting to turn down the inspired partial pressure of the anesthetic as its mixed venous partial pressure increased, Dr. Snow reduces the inspired isoflurane level to 0.8% while keeping the nitrous oxide level at 50%. Within 14 minutes, Matthew's pulse and blood pressure rebound.
- 12:35 PM: After a long surgery, Dr. Snow stops the isoflurane and nitrous oxide and turns on pure oxygen for a few minutes.
- 12:45 PM: In less than 10 minutes, Matthew is breathing spontaneously and is able to respond to questions, although he is still somewhat groggy. Matthew's parents are relieved to find him awake and cheerful after more than 5 hours of anesthesia.

## QUESTIONS

1. What determines the rate of induction and recovery from anesthesia, and how does this differ for children as compared to adults?
2. Why is it necessary to reduce the inspired partial pressure of isoflurane some minutes into the procedure (as Dr. Snow initially neglected to do)?
3. What are the advantages of using a mixture of two anesthetics (in this example, nitrous oxide and isoflurane) instead of just one or the other?
4. Why did Dr. Snow give pure oxygen for a few minutes following the cessation of anesthetic administration?

## PHARMACODYNAMICS OF INHALED ANESTHETICS

General anesthetics distribute well to all parts of the body, becoming most concentrated in the fatty tissues. The CNS is the primary site of action of anesthetics. Most likely, loss of consciousness and amnesia ensue from supraspinal action (i.e., action in the brainstem, midbrain and cerebral cortex), while immobility in response to noxious stimuli is caused by depression of both supraspinal and spinal sensory and motor pathways. Different sites in the CNS are differentially affected by general anesthetics, giving rise to the classical stages observed with increasing anesthetic depth (Fig. 14-1).

## THE MINIMUM ALVEOLAR CONCENTRATION (MAC)

To control the depth of anesthesia, the anesthesiologist must control rather precisely the level of anesthetic in the CNS. This level is denoted by the partial pressure of anesthetic in the CNS, also called the **CNS partial pressure,** $P_{CNS}$. (See Box 14-1 for a discussion of partial pressures versus concentrations and Appendix A for a glossary of abbreviations and symbols.) The anesthesiologist maintains $P_{CNS}$ within the de-

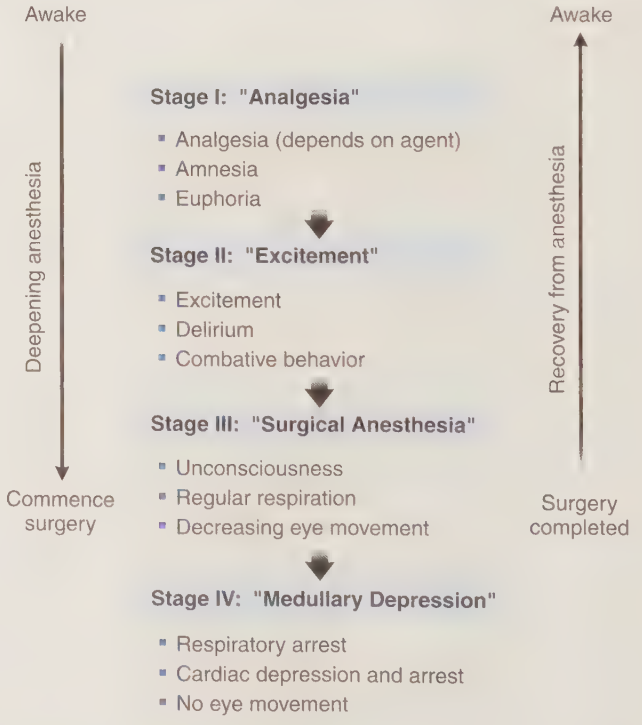


Figure 14-1. The Stages of Anesthesia. The deepening anesthetic state can be divided into four stages, based on observations with diethyl ether. The analgesia of stage I is variable and depends on the particular anesthetic agent. With fast induction, the patient passes rapidly through the undesirable "excitement" phase (stage II). Surgery is generally undertaken in stage III. The anesthesiologist must take care to avoid stage IV, which begins with respiratory arrest. Cardiac arrest occurs later in stage IV. During recovery from anesthesia, the patient progresses through the stages in reverse.

### BOX 14-1. Partial Pressure Versus Concentration

The **partial pressure** of gas A in a mixture of gases is the portion of the total pressure that is supplied by gas A. For ideal gases, the partial pressure of gas A is obtained by multiplying the total pressure by the mole fraction of A in the mixture (i.e., the fraction of molecules in the mixture represented by gas A). The concentration of gas A in the mixture ($[A]_{\text{mixture}}$) is the number of moles of gas A ($n_A$) divided by the volume ($V$); $[A]_{\text{mixture}}$ can also be obtained from the ideal gas equation by dividing the partial pressure of gas $A$ ($P_A$) by the temperature ($T$) and the universal gas constant ($R$).

$$[A]_{\text{mixture}} = n_A / V = P_A / RT$$

Inhaled anesthetics dissolve in the tissues of the body, such as the blood and the brain. The partial pressure of a gas dissolved in a liquid is equal to the partial pressure of free gas in equilibrium with that liquid. For gases, partial pressures are convenient because the partial pressures in all compartments are equal at equilibrium. This is true independent of whether the compartments contain gas that is in the gaseous (alveoli) or the dissolved (tissues) form. In contrast, the concentrations within different compartments will not be equal at equilibrium. To convert the partial pressure of a dissolved gas to its concentration within the solvent, the partial pressure is multiplied by a measure of solubility known as the solvent/gas partition coefficient.

sired range by varying the **inspired partial pressure, $P_I$.** Because the value of $P_{\text{CNS}}$ cannot be monitored directly, it is commonly inferred from the **alveolar partial pressure, $P_{\text{alv}}$.** The alveolar partial pressure is a useful substitute for $P_{\text{CNS}}$, because $P_{\text{CNS}}$ tracks $P_{\text{alv}}$ with only a small time lag (see below). $P_{\text{alv}}$ may be measured directly as the partial pressure of anesthetic in the end-tidal exhaled gas, when the dead space no longer contributes to the exhaled gas.

The alveolar partial pressure that results in the lightest possible anesthesia is termed the **minimum alveolar concentration** (MAC). Specifically, MAC is the alveolar partial pressure that abolishes a movement response to a surgical incision in 50% of patients. The potency of an anesthetic is related inversely to its MAC. If the MAC is small, then the potency is high, and a relatively low partial pressure will be sufficient to cause anesthesia. For example, **isoflurane**—which has a MAC of 0.0114 atm—is much more potent than **nitrous oxide**—which has a MAC of 1.01 atm (Table 14-1).

## THERAPEUTIC AND ANALGESIC INDICES

Loss of response to extremely noxious stimuli, such as intubation, requires a higher partial pressure of anesthetic than is required for loss of response to a simple surgical incision (Fig. 14-2). Still higher partial pressures of anesthetic cause medullary depression. In general, however, anesthetics have steep **dose–response curves** and low therapeutic indices, defined as the ratio of **LD$_{50}$** (the partial pressure that is lethal in 50% of subjects) to MAC (which is analogous to ED$_{50}$; see Chapter 2, Pharmacodynamics). Furthermore, the variability among patients in their response to a given dose of anesthetic is small. Therefore, for all patients, the levels of anesthetic that cause respiratory and cardiac arrest are not much higher than the levels that cause general anesthesia. It should also be noted that no pharmacologic antagonists of general anesthetics exist to correct inadvertently high levels of anesthetic. Although these disadvantages are partly offset by the ability to control $P_{\text{CNS}}$ through control of $P_I$, the combination of low therapeutic index and lack of antagonist means that anesthetics are dangerous drugs that demand specialty training for their proper and safe administration.

Pain relief (analgesia) may or may not occur at a partial pressure lower than that required for surgical anesthesia. The partial pressure at which 50% of persons lose nociception is the AP$_{50}$ (partial pressure that results in analgesia in 50% of patients), and the **analgesic index** is the ratio of MAC to AP$_{50}$. A high analgesic index implies that analgesia is induced at a partial pressure of anesthetic significantly lower than that required for surgical anesthesia. Nitrous oxide, for example, has a higher analgesic index and is a good analgesic; whereas, **halothane** has a lower index and is a poor analgesic.

## THE MEYER–OVERTON RULE

The potency of an anesthetic can be predicted from its physicochemical characteristics. The most reliable predictor has been the anesthetic's solubility in olive oil (or in another lipophilic solvent, such as octanol), as denoted by the **oil/gas partition coefficient, λ(oil/gas)** (Box 14-2). Specifically, *the potency of an anesthetic increases as its solubility in oil increases.* That is, *as λ (oil/gas) increases, MAC decreases.*

The relationship between MAC and λ(oil/gas) is such that MAC multiplied by λ(oil/gas) is nearly constant, independent of the identity of the anesthetic. Because multiplication of the partition coefficient by the partial pressure yields the concentration of anesthetic (Box 14-2), this is equivalent to saying that, at 1 MAC, the concentration of anesthetic in a lipophilic solvent (such as olive oil) is nearly constant for all anesthetics. Thus, the MAC, which varies with the identity of the anesthetic, is actually the partial pressure required to generate a particular concentration of anesthetic in a lipophilic medium such as lipid bilayers in the central nervous system. This correlation, known as the **Meyer–Overton Rule,** correctly holds over at least five orders of magnitude of varying anesthetic solubilities (Fig. 14-3). The constant that represents the concentration of anesthetic at 1 MAC is 1.3 liters of gas per liter of oil ($L_{\text{gas}} / L_{\text{oil}}$), or 0.05 M after dividing by the volume of one mole (see Box 14-2). Thus, if one knows the oil/gas partition coefficient of an anesthetic, one can estimate its MAC from the following equation (see also Table 14-1):

$$\text{MAC} \approx 1.3/\lambda(\text{oil/gas}) \qquad \text{Equation 14-1}$$

## PHARMACOKINETICS OF INHALED ANESTHETICS

A cardiopulmonary model of the **uptake** of anesthetic from the alveoli into the circulation and **distribution** of anesthetic from the circulation to the tissues allows determination

**TABLE 14-1** Properties of Inhaled Anesthetics

| | | SOLVENT/GAS PARTITION COEFFICIENTS | | CONCENTRATION IN OIL AT 1 MAC |
|---|---|---|---|---|
| ANESTHETIC | MAC (atm) | λ(oil/gas) ($L_{gas}$ $L_{tissue}^{-1}$ $atm^{-1}$) | λ(blood/gas) ($L_{gas}$ $L_{tissue}^{-1}$ $atm^{-1}$) | λ(oil/gas) × MAC ($L_{gas}$ $L_{tissue}^{-1}$) |
| **Nitrous oxide** | 1.01 | 1.4 | 0.47 | 1.4 |
| **Desflurane** | 0.06 | 19 | 0.45 | 1.1 |
| **Sevoflurane** | 0.02 | 51 | 0.65 | 1.0 |
| **Diethyl ether** | 0.019 | 65 | 12 | 1.2 |
| **Enflurane** | 0.0168 | 98 | 1.8 | 1.6 |
| **Isoflurane** | 0.0114 | 98 | 1.4 | 1.1 |
| **Halothane** | 0.0077 | 224 | 2.3 | 1.7 |

The commonly used inhaled anesthetics are listed in order of increasing potency (or decreasing MAC). Also listed are the important solvent/gas partition coefficients λ(oil/gas) and λ(blood/gas). λ(oil/gas) defines the potency of the anesthetic (larger is more potent), while λ(blood/gas) defines the rate of induction and recovery of anesthesia (smaller is faster). The product of λ(oil/gas) and MAC for these anesthetics has a rather constant value of 1.3 $L_{gas}$ $L_{tissue}^{-1}$ (with a standard deviation of 0.27). This is an illustration of the Meyer–Overton Rule; another illustration of the rule is shown in Figure 14-3. Also note the general trend that anesthetics with larger λ(oil/gas) tend to have larger λ(blood/gas); this means that there is frequently a trade-off between potency and speed of induction among the inhaled anesthetics. The structures of these agents are shown in Figure 14-14.

of the rate at which the partial pressure of anesthetic rises within the CNS. The anesthesiologist must navigate the small space between a waking patient and causing medullary depression by predicting the effects of various physiologic responses and disease states on the depth of anesthesia. For example, an understanding of the distribution characteristics of anesthetics enabled Dr. Snow to respond appropriately to Matthew's hypotension by lowering the $P_I$ of isoflurane without overcorrecting and causing him to awaken.

The anesthesiologist must also be aware of how anesthetics differ in their pharmacokinetics. The pharmacokinetic characteristics of an ideal general anesthetic would be such that the anesthetic provides a rapid and pleasant induction of surgical anesthesia, followed by a smooth and rapid recovery to a fully functional and conscious state. The pharmacoki-

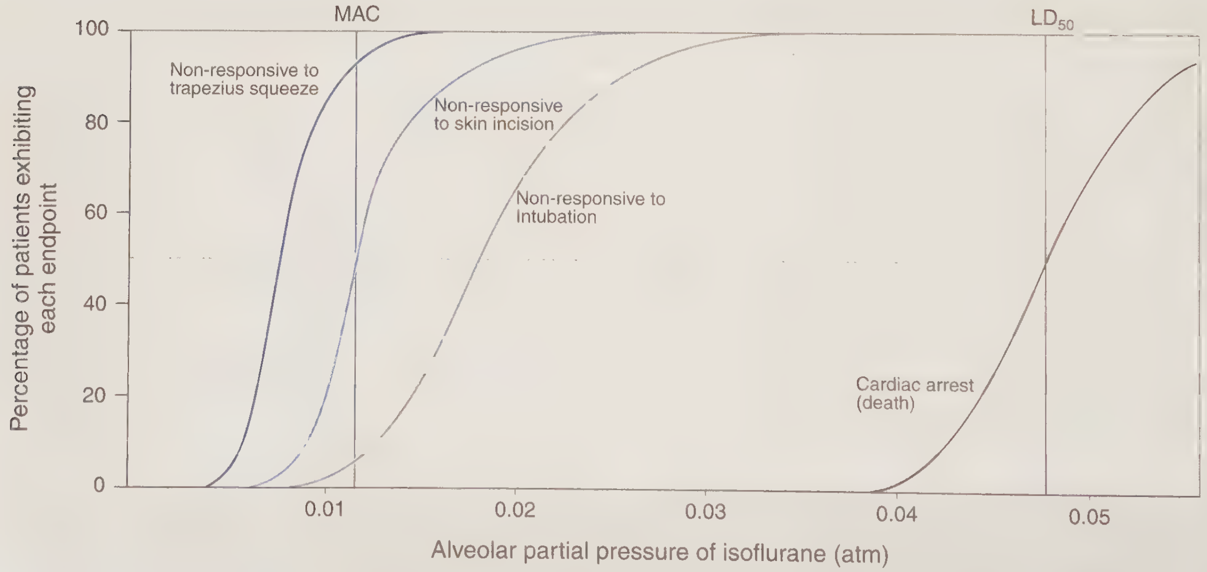


**Figure 14-2. Isoflurane Dose-Response Curves for Various Endpoints.** These curves depict the percentage of patients exhibiting endpoints of nonresponsiveness to a set of stimuli and of cardiac arrest as the alveolar partial pressure of isoflurane is increased. Note that the dose–response curves are quite steep, especially for mild stimuli, and that higher partial pressures are required to achieve lack of response to stronger stimuli. In the example shown, lack of response to intubation in 50% of patients requires nearly 0.02 atm isoflurane, while lack of response to a squeeze of the trapezius muscle requires only 0.008 atm. The MAC is defined as the alveolar partial pressure at which 50% of patients do not respond to a skin incision. The therapeutic index is defined as the $LD_{50}$ divided by the MAC. The theoretical curve for cardiac arrest is derived from a known therapeutic index of about 4 for isoflurane. Accordingly, the anesthesiologist must carefully monitor each individual patient to achieve the desired effect and to avoid cardiac depression.

### BOX 14-2. Partition Coefficients

The **solvent/gas partition coefficient, $\lambda$(solvent/gas),** defines the solubility of a gas in a solvent or, in other words, how the gas "partitions" between its gaseous state and the solution. More specifically, $\lambda$(solvent/gas) is the ratio of the amount of gas dissolved in a given volume of solvent to the amount of free gas that would occupy the same volume of space, all at standard temperature (25°C) and pressure (1 atm) (STP). The solvent could be olive oil, blood, or brain tissue, for example.

Dissolved amounts of gas are typically given not in terms of moles but in terms of the volume that the gas would occupy at STP in a gaseous state. Recall that, to convert from moles to liters at STP, one multiplies by the volume of one mole of gas at 25°C and 1 atm—i.e., by 24.5 L/mol. Thus, $\lambda$(solvent/gas) is the number of liters (L) of gas that will dissolve in one liter of solvent per atmosphere of partial pressure. (Note that the units of $\lambda$(solvent/gas) are $L_{\text{gas}}\ L_{\text{solvent}}^{-1}\ \text{atm}^{-1}$, or simply $\text{atm}^{-1}$.)

For a particular solvent, a gas with a larger $\lambda$(solvent/gas) is more soluble in that solvent. For example, diethyl ether has a $\lambda$(blood/gas) of about 12 $L_{\text{diethyl ether}}\ L_{\text{blood}}^{-1}\ \text{atm}^{-1}$, so diethyl ether is relatively soluble in blood. In contrast, nitrous oxide has a $\lambda$(blood/gas) of about 0.47 $L_{\text{nitrous oxide}}\ L_{\text{blood}}^{-1}\ \text{atm}^{-1}$, so nitrous oxide is relatively insoluble in blood (see Table 14-1 and Figure 14-8, for an example).

Likewise, a gas may have different solubilities in different solvents. Solvents or tissues in which a gas has a large partition coefficient (high solubility) will dissolve large amounts of the gas at a given partial pressure, resulting in a high concentration of the gas in that solvent or tissue. Thus, large amounts of gas must be transferred to change the partial pressure by an appreciable amount. In contrast, solvents or tissues in which a gas has a small partition coefficient (low solubility) will dissolve only small amounts of the gas at a given partial pressure. In this case, transferring a small amount of the gas will significantly change the partial pressure (Fig. 14-8).

For any given partial pressure, Henry's Law for dilute solutions allows the concentration of gas A in a solvent ($[A]_{\text{solution}}$) to be calculated from $\lambda$(solvent/gas). The partial pressure is multiplied by the partition coefficient to calculate the concentration in terms of $L_{\text{gas}}$ per $L_{\text{solvent}}$. The result is divided by the volume of one mole of gas at 25°C at 1 atm (24.5 L/mol) to get the molar concentration.

$$\begin{aligned}[A]_{\text{solution}} &= P_{\text{solvent}} \times \lambda\,(\text{solvent/gas}) \quad \{\text{in terms of } L_{\text{gas}}/L_{\text{solvent}}\}\\ &= P_{\text{solvent}} \times \lambda\,(\text{solvent/gas})\,/(24.5\ \text{L/mol})\\ &\qquad\qquad \{\text{in terms of mol}_{\text{gas}}/L_{\text{solvent}}\}\end{aligned}$$

For example, because the $\lambda$(blood/gas) of nitrous oxide is 0.47 $L_{\text{nitrous oxide}}\ L_{\text{blood}}^{-1}\ \text{atm}^{-1}$, if the partial pressure of nitrous oxide in the blood is 0.50 atm, then the concentration is $0.50\ \text{atm} \times 0.47\ L_{\text{nitrous oxide}}\ L_{\text{blood}}^{-1}\ \text{atm}^{-1} = 0.24\ L_{\text{nitrous oxide}}\ L_{\text{blood}}^{-1}$ or 9.6 mM (after dividing by 24.5 L/mol). Also note that doubling the partial pressure will double the concentration.

A partition coefficient can also be defined for the partioning of a gas between two solvents. For example, the tissue/blood partition coefficient, $\lambda$(tissue/blood), is the ratio of the molar concentration of gas in the tissue ($[A]_{\text{tissue}}$) to the molar concentration of gas in the blood ($[A]_{\text{blood}}$) at equilibrium (note that this coefficient is unitless). From the above equation defining concentration and the fact that partial pressures are equal at equilibrium, it follows that:

$$\begin{aligned}\lambda\,(\text{tissue/blood}) &= [A]_{\text{tissue}}\,/\,[A]_{\text{blood}}\\ &= \lambda\,(\text{tissue/gas})\,/\,\lambda\,(\text{blood/gas})\end{aligned}$$

netics of individual agents are discussed below; this section deals with general principles of the **uptake model,** which uses basic respiratory and cardiovascular physiology to predict the pharmacokinetics of the inhaled anesthetics. As discussed below, the uptake model depends on calculations of the time required for the equilibration of anesthetic partial pressures in the tissues with the inspired anesthetic partial pressure.

## CONCEPTS FROM RESPIRATORY PHYSIOLOGY

### Local Equilibration

During general anesthesia, the patient breathes, either spontaneously or via a ventilator, an anesthetic or mixture of anesthetics, and oxygen and/or normal air. Once the anesthetic gas reaches the alveoli, it must diffuse across the respiratory epithelium into the alveolar capillary bed. According to Fick's law, the **rate of diffusion** of gas through a sheet of tissue down its partial pressure gradient is proportional to the tissue area and the partial pressure difference between the two sides, and is inversely proportional to the thickness of the sheet:

$$\text{Diffusion rate} = D \times (A/l) \times P \qquad \text{Equation 14-2}$$

where $D$ = diffusion constant; $A$ = surface area; $l$ = thickness; and $P$ = partial pressure difference.

One principal evident from Fick's Law is that the equalization of the partial pressure of the gas, not its concentration, defines the approach to equilibrium across a boundary sheet. Thus, at equilibrium (i.e., when the net diffusion rate is zero), the partial pressure in the two compartments is the same, even though the concentration in the two compartments may be different.

The lung optimizes the rate of gas diffusion with its enormous alveolar surface area (~75 $m^2$, or nearly half a tennis court) and thin epithelium (~0.3 μm, which is less than 1/20th the diameter of a red blood cell). Accordingly, the alveolar partial pressure $P_{\text{alv}}$ and the systemic arterial partial pressure $P_{\text{art}}$ are nearly the same at all times (small amounts of physiologic shunting keep $P_{\text{art}}$ slightly lower than $P_{\text{alv}}$). By using the lungs as an uptake system for inhaled anesthetics, anesthesiologists take advantage of the body's system for absorbing oxygen.

Similarly, the capillary beds in tissues have evolved to deliver oxygen rapidly to all cells in the body. The distances between arterioles are small, and diffusion pathways are of

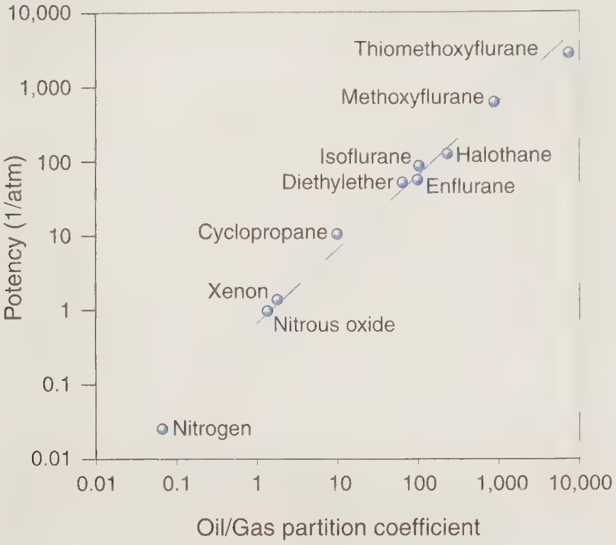


**Figure 14-3. The Meyer-Overton Rule.** Molecules with a larger oil/gas partition coefficient (λ(oil/gas)) are more potent general anesthetics. This log–log plot shows the very tight correlation between lipid solubility (λ(oil/gas)) and anesthetic potency over five orders of magnitude. Note that even such gases as xenon and nitrogen can act as general anesthetics when breathed at high enough partial pressures. The equation describing the line is: Potency = λ(oil/gas) / 1.3. Recall that Potency = 1/MAC.

the order of one cell diameter. Consequently, the arterial partial pressure of a general anesthetic can equilibrate completely with tissues in the time required for blood to traverse the capillary bed. Likewise, the partial pressure in the postcapillary venules $P_{\text{venule}}$ equals the partial pressure in the tissue $P_{\text{tissue}}$.

Another way of stating the above conclusion is that *the transfer of anesthetic in both the lungs and the tissues is limited by perfusion rather than diffusion.* Because perfusion is rate-limiting, increasing the rate of diffusion (e.g., by using a lower molecular weight anesthetic) will not, by itself, increase the rate of induction of anesthesia.

## Global Equilibration

If an anesthetic is inspired for a long enough period of time, all compartments in the body will equilibrate to the same partial pressure (equal to $P_I$). This global equilibration may be divided into a series of partial pressure equilibrations between each successive compartment and its incoming flow of anesthetic. In the case of the tissues, the incoming flow is the arterial blood flow, with partial pressure approximately equal to $P_{\text{alv}}$. In the case of the alveoli, the incoming flow is the alveolar ventilation with partial pressure $P_I$.

The **time constant** τ describes the rate of approach of a compartment's partial pressure to that of its incoming flow. Specifically, τ is the time required for equilibration to be 63% complete. This time constant is convenient because it can be calculated by dividing the compartment's **volume capacity** (relative to the delivering medium; see below) by the **flow rate.** In other words, once a volume of flow equal to the capacity of a compartment has gone through that compartment, the partial pressure of anesthetic in the compartment (i.e., in the tissues or alveoli) will be 63% of the partial pressure in the incoming flow (i.e., in the arterial blood flow or alveolar ventilation, respectively). Equilibration is 95% complete after three time constants.

$$\tau = \text{Volume Capacity/Flow Rate} \qquad \text{Equation 14-3}$$

$$P_{\text{compartment}} = P_{\text{flow}}\left[1 - e^{-(t/\tau)}\right]$$

$$\text{where } t = \text{elapsed time} \qquad \text{Equation 14-4}$$

These equations describe what should make intuitive sense: equilibration of the partial pressure of the compartment with the incoming flow takes place more quickly (i.e., the time constant is smaller) when the inflow is larger or the compartment capacity is smaller.

## THE UPTAKE MODEL

For simplicity, the model of anesthetic uptake and distribution organizes the tissues of the body into groups based on similar characteristics. Each group can be modeled as a container with a particular capacity for anesthetic and a particular level of blood flow delivering anesthetic. An adequate approximation groups the tissues into three main compartments that are perfused in parallel (Fig. 14-4). The **vessel rich group (VRG),** which consists of the central nervous system and visceral organs, has a low capacity and high flow. The **muscle group (MG),** which consists of muscle and skin, has a high capacity and moderate flow. The **fat group (FG)** has a very high capacity and low flow. (A fourth group, the **vessel poor group (VPG),** which consists of bone, cartilage, and ligaments, has a negligible flow and capacity, and its omission does not significantly affect the model.)

The rate of increase of the partial pressure in the VRG ($P_{\text{VRG}}$) is of the greatest interest because the VRG includes the CNS. The overall equilibration of $P_{\text{VRG}}$ with the inspired partial pressure occurs in two steps, either of which may be rate limiting. First, the alveolar and inspired partial pressures equilibrate ($P_{\text{alv}}$ approaches $P_I$, or $P_{\text{alv}} \rightarrow P_I$). Second, $P_{\text{VRG}}$ (and specifically $P_{\text{CNS}}$) equilibrates with the arterial partial pressure (which is essentially equal to the alveolar partial pressure) ($P_{\text{VRG}} \rightarrow P_{\text{art}}$). The discussion will now consider the time constant for each of these two steps, and define conditions under which one or the other is rate limiting.

### Equilibration of Alveolar with Inspired Partial Pressure

The equilibration of $P_{\text{alv}}$ with $P_I$ is conceptually the first step of the equilibration of $P_{\text{VRG}}$ with $P_I$. During induction of anesthesia, $P_{\text{VRG}}$ can never be higher than $P_{\text{alv}}$; if $P_{\text{alv}}$ rises slowly, then $P_{\text{VRG}}$ must also rise slowly.

To calculate the time constant for the approach of $P_{\text{alv}}$ to $P_I$, $\tau\{P_{\text{alv}} \rightarrow P_I\}$, the flow rate and volume capacity must be defined. The delivering medium is free gas arriving through the airways, and the compartment is the lung and alveoli. The volume capacity is simply the volume of gas that remains in the lungs after normal exhalation, or the **functional residual capacity** (FRC, typically ~3 L for an average adult). Assume initially that the only component of the flow rate is the rate of

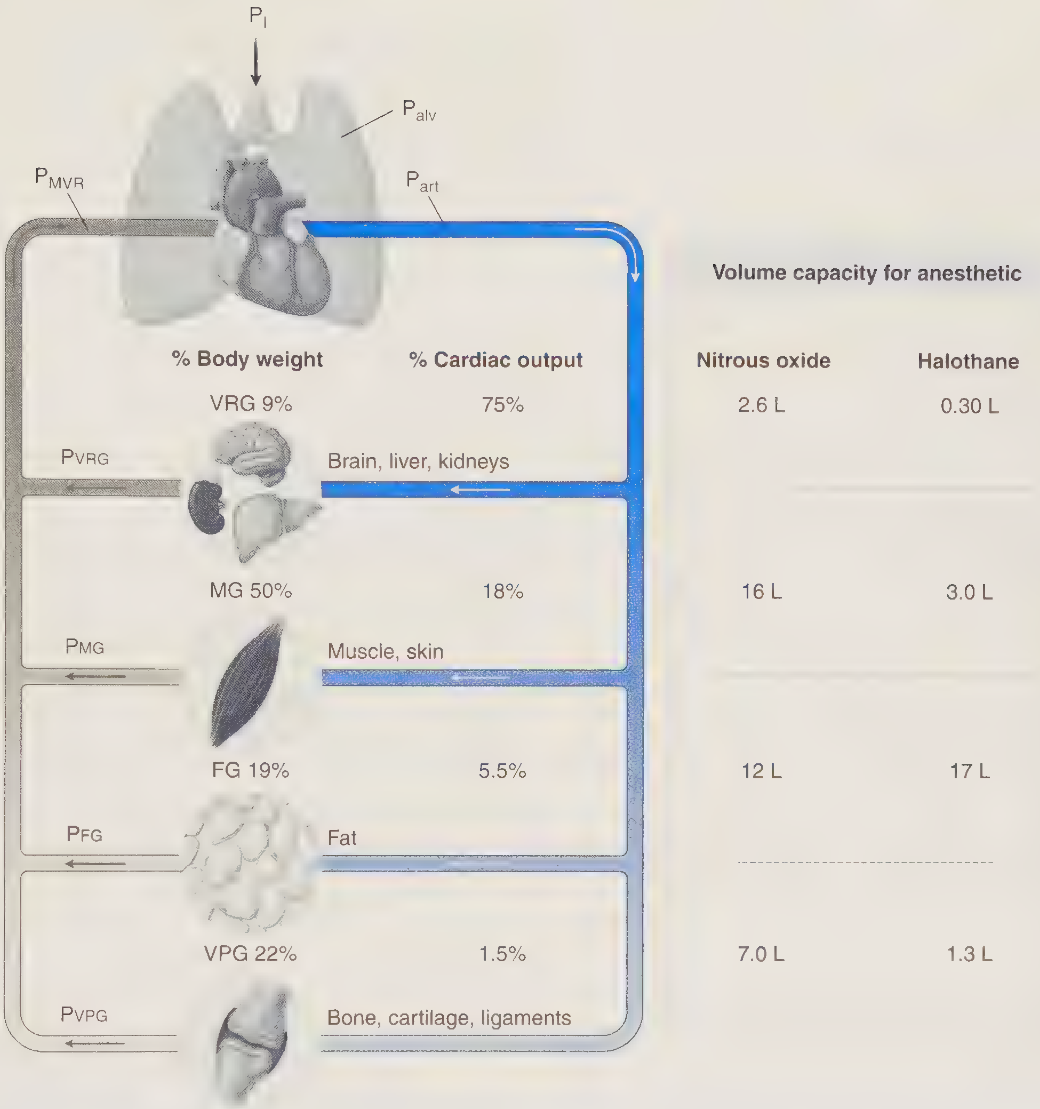


**Figure 14-4. Distribution of Cardiac Output and Volume Capacity for General Anesthetics among the Major Tissue Compartments.** The tissues of the body can be divided into four groups based on their level of perfusion and their capacity to take up anesthetic. These include the Vessel Rich Group (VRG), Muscle Group (MG), Fat Group (FG), and Vessel Poor Group (VPG). (The contribution of the VPG is generally ignored in most pharmacokinetic models of anesthesia.) The VRG, which contains the internal organs including the brain, constitutes a small percentage of the total body weight (9%), has the lowest capacity for anesthetics, and receives most of the cardiac output (75%). The high perfusion and low capacity allows $P_{VRG}$ to equilibrate rapidly with $P_{art}$. Also, the VRG makes the largest contribution to the mixed venous return partial pressure $P_{MVR}$, which is equal to ($0.75\ P_{VRG} + 0.18\ P_{MG} + 0.055\ P_{FG} + 0.015\ P_{VPG}$).

**alveolar ventilation,** which delivers the anesthetic ($V_{alv}$ = {Tidal Volume – Dead Space} × Respiratory Rate; for an average adult, $V_{alv} = \{0.5\text{ L} - 0.125\text{ L}\} \times 16\text{ min}^{-1} \approx 6\text{ L/min}$). Then, because

$$\tau\{P_{alv} \rightarrow P_I\} = \text{FRC}/V_{alv} \qquad \text{Equation 14-5}$$

a typical value for $\tau\{P_{alv}\rightarrow P_I\}$ is 3 L / 6 L/min, or 0.5 min—independent of the particular gas being inhaled. In children, the increased alveolar ventilation rate and decreased FRC (smaller lungs) both tend to shorten the time constant and to accelerate equilibration between the alveolar and inspired partial pressures.

The assumption to this point has been that no uptake of anesthetic into the bloodstream occurs, as would be the case if the solubility of the anesthetic in blood were zero. In practice, at the same time that alveolar ventilation is delivering anesthetic to the alveoli, anesthetic is also being removed from the alveoli by diffusion into the bloodstream. The balance between delivery and removal is analogous to adding water into a leaky bucket (Fig. 14-5). The level of water in the bucket (which represents the alveolar partial pressure) is determined both by the rate at which the water is added (the minute ventilation) and the size of the leak (the rate of anesthetic uptake from the alveoli into the bloodstream). Increasing anesthetic delivery; for example, by using a higher ventilation rate or a higher inspired partial pressure, will increase the alveolar partial pressure of the gas, just as adding water faster will increase the level of water in the bucket. Conversely, increasing anesthetic removal, for example, by increasing the perfusion rate or using

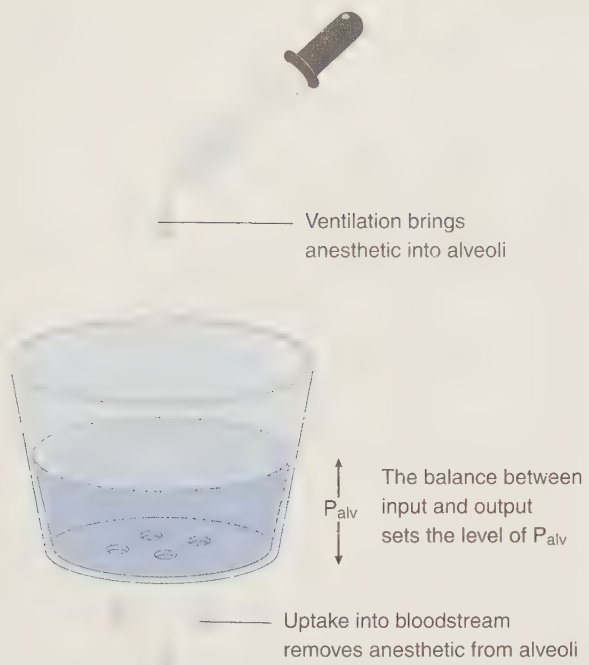

Ventilation brings anesthetic into alveoli
$P_{alv}$
The balance between input and output sets the level of $P_{alv}$
Uptake into bloodstream removes anesthetic from alveoli

**Figure 14-5. Determinants of the Alveolar Partial Pressure of an Inhaled Anesthetic.** The alveolar partial pressure, represented by the depth of fluid in the bucket, results from the balance between delivery by ventilation and removal by uptake into the bloodstream. Increased delivery of anesthetic, resulting from either increased ventilation or an increased inspired partial pressure of anesthetic, raises $P_{alv}$. In contrast, increased uptake into the bloodstream, caused by a large λ(blood/gas) or increased cardiac output, lowers $P_{alv}$.

a more blood-soluble anesthetic, will decrease the alveolar partial pressure of the gas; this is analogous to increasing the size of the leak in the bucket. Thus, uptake of anesthetic from the alveoli into the bloodstream constitutes a negative component to the flow (i.e., a flow out of the lungs) that makes the time constant longer than the theoretical case where $\tau\{P_{alv} \rightarrow P_I\}$ equals FRC divided by $V_{alv}$.

The magnitude of the increase in the time constant compared to the limiting case depends on the rate of uptake of anesthetic by the blood, with longer $\tau\{P_{alv} \rightarrow P_I\}$ resulting from greater uptake. If one knows the cardiac output (i.e., the volume of blood pumped by the heart in 1 minute) and the value of the instantaneous difference between the pulmonary arterial partial pressure (which equals the systemic partial pressure of the mixed venous return, $P_{MVR}$) and the pulmonary venous partial pressure (which equals the systemic arterial partial pressure, $P_{art}$), then one can calculate the rate of uptake of a gas from the alveoli:

$$\text{Rate of uptake } \{\text{in L}_{gas}/\text{min}\} = \lambda(\text{blood/gas}) \times (P_{art} - P_{MVR}) \times \text{CO} \qquad \text{Equation 14-6}$$

where CO = cardiac output in liters of blood per minute. Equation 14-7 follows from Equation 14-6 because the anesthetic concentration $[A]_{blood}$ is equal to λ(blood/gas) $P_{blood}$ (see Box 14-2):

$$\text{Rate of uptake } = ([A]_{art} - [A]_{MVR}) \times \text{CO} \qquad \text{Equation 14-7}$$

If any of the components of these equations approaches zero, the rate of uptake becomes small, and the delivery of anesthetic by ventilation is free to drive the alveolar partial pressure toward the inspired partial pressure. In other words, equilibration of alveolar with inspired partial pressure is faster (i.e., $\tau\{P_{alv} \rightarrow P_I\}$ is smaller) with lower blood solubility of the anesthetic (smaller λ(blood/gas)), lower cardiac output, or smaller arterial (~alveolar) to venous partial pressure difference.

## Equilibration of Tissue with Alveolar Partial Pressure

In addition to the equilibration between $P_{alv}$ and $P_I$, equilibration between $P_{tissue}$ and $P_{art}$ (which is nearly equal to $P_{alv}$) must occur for $P_{tissue}$ to equilibrate with $P_I$. Changes in $P_{alv}$ are transmitted rapidly to systemic arterioles, because equilibration across the pulmonary epithelium is fast, and the circulation time from pulmonary veins to tissue capillaries is generally less than 10 seconds. Thus, the time constant for equilibration between $P_{tissue}$ and $P_{alv}$ can be approximated as the time constant for equilibration between $P_{tissue}$ and $P_{art}$. To calculate the time constant $\tau\{P_{tissue} \rightarrow P_{art}\}$, one must define the capacity of the compartment and the flow rate of the delivering medium. The flow rate is simply the rate at which blood perfuses the tissue. Recall that capacity is a volume capacity relative to the delivering medium. Specifically, *the capacity is the volume that the tissue would need to contain all of its gas if the solubility of the gas in the tissue were the same as that in the blood.* (This definition is similar to that of the volume of distribution of a drug; see Chapter 3):

$$\text{Relative Volume Capacity of Tissue} = ([A]_{tissue} \times \text{Vol}_{tissue})/[A]_{blood} \qquad \text{Equation 14-8}$$

where $\text{Vol}_{tissue}$ is the volume of tissue. Equation 14-9 follows from Equation 14-8 because $[A]_{tissue} / [A]_{blood}$ at equilibrium is equal to λ(tissue/blood) (see Box 14-2):

$$\text{Relative Volume Capacity of Tissue} = \lambda(\text{tissue/blood}) \times \text{Vol}_{tissue} \qquad \text{Equation 14-9}$$

Then, using Equation 14-3, we can write

$$\tau\{P_{tissue} \rightarrow P_{art}\} \approx \tau\{P_{tissue} \rightarrow P_{alv}\} = \text{Relative Volume Capacity of Tissue}/Q_{tissue} \qquad \text{Equation 14-10}$$

$$\tau\{P_{tissue} \rightarrow P_{art}\} = \lambda(\text{tissue/blood}) \times \text{Vol}_{tissue}/Q_{tissue} \qquad \text{Equation 14-11}$$

where $Q_{tissue}$ is tissue perfusion in L/min.

The tissue groups differ greatly in their capacities for anesthetic and in the time constants for their equilibration with arterial (and thus alveolar) partial pressure. With a small λ(tissue/blood) (Table 14-2) and a small volume (~6 L), the VRG has a low capacity for anesthetic. The combination of low capacity and high blood flow (75% of cardiac output) results in a very short equilibration time constant ($\tau\{P_{VRG} \rightarrow P_{alv}\}$) for the VRG. With a slightly higher λ(tissue/blood), a much larger volume (~33 L), and only moderate blood flow, the MG

### TABLE 14-2 Tissue/Blood Partition Coefficients

| ANESTHETIC | TISSUE/BLOOD PARTITION COEFFICIENTS<br>$\lambda$(brain/blood) (unitless) | $\lambda$(muscle/blood) (unitless) | $\lambda$(fat/blood) (unitless) |
|---|---|---|---|
| **Nitrous oxide** | 1.1 | 1.2 | 2.3 |
| **Diethyl ether** | 2.0 | 1.3 | 5 |
| **Desflurane** | 1.3 | 2.0 | 27 |
| **Enflurane** | 1.4 | 1.7 | 36 |
| **Isoflurane** | 1.6 | 2.9 | 45 |
| **Sevoflurane** | 1.7 | 3.1 | 48 |
| **Halothane** | 1.9 | 3.4 | 51 |

The tissue/blood partition coefficient describes the comparative solubility of an anesthetic in a tissue compared to blood. λ(tissue/blood) is obtained from the ratio of the concentration of anesthetic in the tissue to the concentration in the blood at equilibrium (i.e., when the partial pressure is the same in both tissues). Alternatively, one may take advantage of the fact that λ(tissue/blood) = λ(tissue/gas) / λ(blood/gas) (see Box 14-2). With very few minor exceptions, the general trend is λ(fat/blood) >> λ(muscle/blood) > λ(brain/blood). High values of λ(fat/blood) for the inhaled anesthetics give the FG a very high capacity for anesthetics.

has a longer equilibration time constant ($\tau\{P_{MG}\rightarrow P_{art}\}$). Finally, with an extremely high λ(tissue/blood), a large volume, and low blood flow, the FG has an extremely long equilibration time constant ($\tau\{P_{FG}\rightarrow P_{art}\}$) (Table 14-3 and Fig. 14-6).

Because the anesthesiologist seeks to control $P_{CNS}$, the time constant for equilibration of the brain partial pressure $P_{brain}$ with the arterial partial pressure $P_{art}$ (which is nearly equal to $P_{alv}$) is of particular interest. The volume of the brain is approximately 1.4 L, the blood flow to the brain is about 0.9 L/min, and an average λ(brain/blood) for most anesthetics is about 1.6. Then, because

$$\text{Relative Volume Capacity of Brain} = \lambda(\text{brain/blood}) \times \text{Vol}_{\text{brain}} \quad \text{Equation 14-12}$$

$$\tau\{P_{\text{brain}} \rightarrow P_{\text{art}}\} = \lambda(\text{brain/blood}) \times \text{Vol}_{\text{brain}}/Q_{\text{brain}}$$
$$\tau\{P_{\text{brain}} \rightarrow P_{\text{art}}\} = (1.6 \times 1.4\ \text{L})/(0.9\ \text{L/min})$$
$$= 2.5\ \text{min} \quad \text{Equation 14-13}$$

where $\text{Vol}_{\text{brain}}$ is the volume of the brain, and $Q_{\text{brain}}$ is the blood flow to the brain.

Variations in λ(brain/blood) among the different anesthetic agents causes $\tau\{P_{brain}\rightarrow P_{art}\}$ to range from 1.7 min for nitrous oxide [λ(brain/blood) = 1.1] to 3.1 min for diethyl ether [λ(brain/blood) = 2.0] (Table 14-2). Of course, variability in blood flow to the brain also affects $\tau\{P_{brain}\rightarrow P_{art}\}$. In summary, *the time constant for equilibration of the CNS with the alveolar partial pressure is short, and relatively independent of the particular anesthetic being used.*

### TABLE 14-3 Time Constants for Equilibration of Tissue with Arterial Partial Pressure

| ANESTHETIC | TIME CONSTANT FOR EQUILIBRATION OF TISSUE WITH ARTERIAL PARTIAL PRESSURE, $\tau\{P_{tissue}\rightarrow P_{art}\}$<br>VRG (min) | MG (min) | FG (min) |
|---|---|---|---|
| **Nitrous oxide** | 1.5 | 36 | 104 |
| **Diethyl ether** | 2.7 | 39 | 227 |
| **Desflurane** | 1.7 | 61 | 1223 |
| **Enflurane** | 1.9 | 51 | 1631 |
| **Isoflurane** | 2.1 | 88 | 2039 |
| **Sevoflurane** | 2.3 | 94 | 2175 |
| **Halothane** | 2.5 | 103 | 2311 |

The time constants $\tau\{P_{tissue}\rightarrow P_{art}\}$ describe the time for 63% equilibration of the tissue with arterial (and therefore alveolar) partial pressure. Notice the very small time constants for equilibration of the VRG, in contrast to the large time constants for MG equilibration and very large time constants for FG equilibration. For all anesthetics except nitrous oxide, the partial pressure of the FG remains far below that of the alveolus for even the longest surgical procedures. Conversely, the VRG partial pressure is nearly in equilibrium with the alveolus from almost the start of anesthetic administration. The values in this table were calculated from the equation $\tau\{P_{tissue}\rightarrow P_{art}\}$ = λ(tissue/blood) × Volume of tissue / Blood flow to tissue.

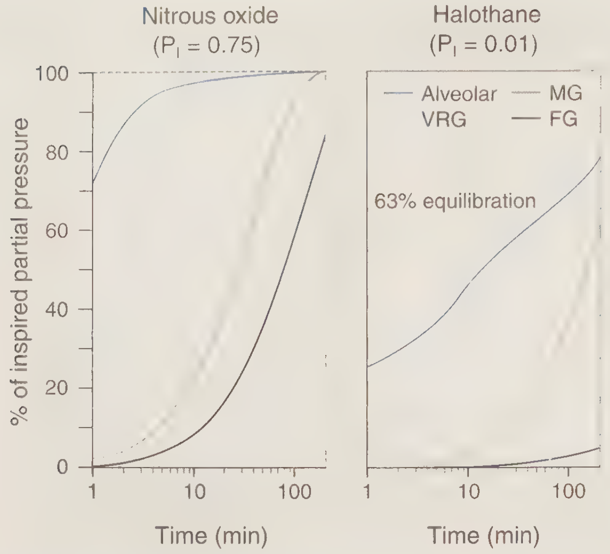


**Figure 14-6. Equilibration of the Tissue Groups with the Inspired Partial Pressure.** These curves show, as a function of time, the approach of the partial pressures in the alveoli and in the three major tissue groups toward the inspired partial pressure. The partial pressure in the VRG equilibrates rapidly with the alveolar partial pressure, while the MG equilibrates more slowly, and the FG much more slowly. For a ventilation-limited anesthetic such as halothane, the rate at which the partial pressure in the VRG rises is limited not by its approach to the alveolar partial pressure, but rather by the rise of the alveolar toward the inspired partial pressure. In other words, the rate-limiting step is the equilibration of the alveolar partial pressure with the inspired partial pressure. In contrast, for a perfusion-limited anesthetic such as nitrous oxide, the alveolar partial pressure rises so quickly that the rate of rise of the VRG partial pressure is as much limited by its rise toward the alveolar partial pressure as by the rise of $P_{alv}$ toward $P_I$. The dashed line shows the point at which the partial pressure is 63% of $P_I$; the time constant for equilibration of each tissue group with $P_I$ is approximated by the time at which each curve crosses this line.

## The Rate-Limiting Step

As described above, the equilibration of the CNS with the inspired partial pressure occurs in two steps. Unlike $\tau\{P_{brain} \rightarrow P_{art}\}$, which is relatively independent of the particular anesthetic being used, $\tau\{P_{alv} \rightarrow P_I\}$ varies greatly among different anesthetics. On this basis, inhaled anesthetics can be divided into two broad categories:

- ventilation-limited anesthetics, such as diethyl ether, **enflurane,** isoflurane; and halothane; and
- perfusion-limited anesthetics, such as nitrous oxide, **desflurane,** and **sevoflurane**.

**Ventilation-limited anesthetics** have a long, rate-limiting $\tau\{P_{alv} \rightarrow P_I\}$ because of their high λ(blood/gas): the high rate of uptake of anesthetic into the bloodstream prevents $P_{alv}$ from rising rapidly. Thus, the slow and rate-limiting equilibration of alveolar with inspired partial pressure results in slow induction of anesthesia and slow recovery from anesthesia. Accordingly, for these anesthetics, physiologic or pathologic changes that act to increase the rate of rise of the alveolar partial pressure will speed induction. Conversely, because equilibration of the tissue with the arterial partial pressure is not rate limiting, physiologic or pathologic changes that shorten $\tau\{P_{VRG} \rightarrow P_{art}\}$ will have little effect on induction time (see below).

**Perfusion-limited anesthetics** have a $\tau\{P_{alv} \rightarrow P_I\}$ that is similar in magnitude to $\tau\{P_{VRG} \rightarrow P_{art}\}$ because their λ(blood/gas) is small. Induction and recovery occur quickly, and neither $\tau\{P_{alv} \rightarrow P_I\}$ nor $\tau\{P_{VRG} \rightarrow P_{art}\}$ may be clearly rate limiting. Accordingly, induction time may be affected by changes in either the rate of rise of alveolar partial pressure or the rate at which $P_{CNS}$ approaches $P_{art}$ (e.g., see the discussion of hyperventilation below). Physiologic changes may alter the balance between $\tau\{P_{alv} \rightarrow P_I\}$ and $\tau\{P_{VRG} \rightarrow P_{art}\}$. See Figure 14-6 for a graphic comparison of the kinetics of a ventilation-limited and a perfusion-limited anesthetic.

The characteristic that distinguishes perfusion-limited from ventilation-limited anesthetics is the blood/gas partition coefficient, λ(blood/gas). With the smaller λ(blood/gas) of perfusion-limited anesthetics, the bloodstream removes less anesthetic from the alveoli; thus, the alveolar partial pressure rises more quickly and induction is faster (Fig. 14-7). This is the key point, although the correlation may seem paradoxical at first: *agents that are less soluble in the blood induce anesthesia faster.*

To clarify, consider two hypothetical anesthetics that differ solely in λ(blood/gas): Anesthetic A has a small λ(blood/gas); while Anesthetic B has a large λ(blood/gas). Because Anesthetics A and B are identical in λ(oil/gas), they have the same MAC. They also have identical λ(brain/blood), so their

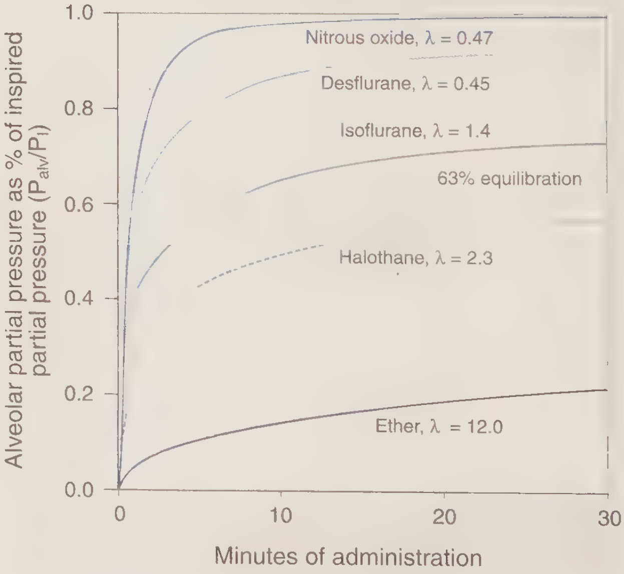


**Figure 14-7. Rate of Approach of the Alveolar Toward the Inspired Partial Pressure.** For agents with smaller λ(blood/gas) such as nitrous oxide, the alveolar partial pressure approaches the inspired partial pressure quickly, while for agents with larger λ(blood/gas) such as ether, the alveolar partial pressure approaches the inspired partial pressure much more slowly. The dashed line shows the point at which $P_{alv}/P_I = 0.63$; the time constant $\tau\{P_{alv} \rightarrow P_I\}$ is approximated by the time at which each curve crosses this line. λ = λ(blood/gas).

$\tau\{P_{brain} \rightarrow P_{alv}\}$ is the same (see Equations 14-12 and 14-13). To cause anesthesia, both must achieve the same partial pressure in the CNS. At any particular partial pressure, however, the blood and CNS contain more moles of Anesthetic B than Anesthetic A because Anesthetic B is more soluble than Anesthetic A in the blood and CNS. The transfer of a larger number of moles of Anesthetic B out of the lungs slows the rate of rise of $P_{alv}$, so a longer period is necessary for Anesthetic B than for Anesthetic A to achieve the anesthetic partial pressure in the CNS (Fig. 14-8).[a]

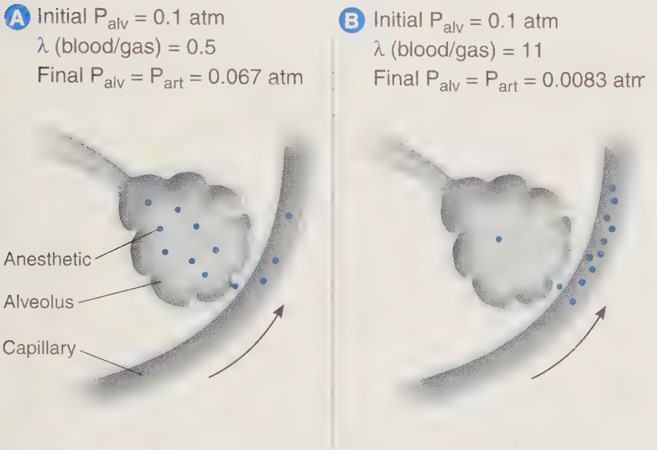

A
Initial $P_{alv}$ = 0.1 atm
λ (blood/gas) = 0.5
Final $P_{alv}$ = $P_{art}$ = 0.067 atm
B
Initial $P_{alv}$ = 0.1 atm
λ (blood/gas) = 11
Final $P_{alv}$ = $P_{art}$ = 0.0083 atr
Anesthetic
Alveolus
Capillary

**Figure 14-8. Why Do Anesthetics with Smaller λ(Blood/Gas) have Faster Induction Times?** Consider two equally potent anesthetics inspired at the same partial pressure, $P_I$. Before any anesthetic molecules have been taken up from the alveolus into the blood, the alveolar partial pressure, $P_{alv}$, of each anesthetic is 0.1 atm. This partial pressure would be represented in the diagram by twelve anesthetic "spheres" in each alveolus. For each anesthetic, equilibration of the partial pressures in the alveolus and the capillary then takes place. For a relatively blood-insoluble agent with λ(blood/gas) = 0.5 (**Anesthetic A,** which closely resembles nitrous oxide, desflurane, sevoflurane, and cyclopropane), the transfer of a small amount of anesthetic from the alveolus significantly raises the partial pressure in the capillary. To illustrate, consider a time, $t_v$, when the volume of blood that has flowed past the alveolar wall is equal to the volume of the alveolus. At that time, the concentration in the alveolus will be twice that in the capillary (because λ(blood/gas) = 0.5; see Box 14-2) when four of the "spheres" have been transferred from the alveolus to the capillary and eight "spheres" remain in the alveolus. The partial pressure in the alveolus will now have dropped to $(8/12) \times 0.1 = 0.067$ atm. This is also the partial pressure in the capillary. In contrast, for a very blood-soluble agent with λ(blood/gas) = 11 (**Anesthetic B,** which closely resembles diethyl ether), much larger amounts of anesthetic must dissolve in the blood to raise the partial pressure in the capillary. Using the same illustration as above, at $t_v$, eleven of the twelve "spheres" will have been transferred from the alveolus to the capillary, and the remaining $P_{alv}$ will be given by $(1/12) \times 0.1 = 0.0083$ atm. Thus, although the inspired partial pressure of the two anesthetics is the same, at time $t_v$, the $P_{alv}$ and $P_{art}$ of Anesthetic A will be 8 times higher than that of Anesthetic B. Within approximately 1 minute, $P_{brain}$ will also have these values. Thus, the brain partial pressure rises toward the inspired partial pressure much more rapidly for Anesthetic A than for Anesthetic B (i.e., the induction time for Anesthetic A is much shorter than that for Anesthetic B). If the reader is confused by the fact that more molecules of Anesthetic B are being carried to the brain, recall that λ(brain/blood) is ~1 for all of the commonly used anesthetics (Table 14-2). Thus, proportionally many more molecules of Anesthetic B than Anesthetic A must be delivered to the brain to raise the partial pressure of each anesthetic by an equivalent amount. See Boxes 14-1 and 14-2 and the Appendix for definitions.

## APPLICATIONS OF THE UPTAKE MODEL

Throughout the following discussion, it is critical to remember that the primary responsibility of the anesthesiologist is to keep the patient well oxygenated and the vital signs stable while manipulating the inspired partial pressure of anesthetic to maintain the desired depth of anesthesia.

Armed with the uptake model, the anesthesiologist can predict the effects of cardiopulmonary changes and pathologic states on the depth of anesthesia. Changes in ventilation and cardiac output may be caused by the general anesthetic itself, by the trauma of surgery, or by some other physiologic or pathophysiologic process.

The effects of changes in both ventilation and cardiac output on $P_{CNS}$ are greatest when the difference between $P_I$ and $P_{alv}$ is greatest; that is, early in the course of anesthesia (Fig. 14-6). To understand this, consider the partial pressure in the mixed venous return (MVR), $P_{MVR}$, which is a weighted average of the partial pressures in each of the tissue groups, with $P_{VRG}$ making the largest contribution because the VRG receives the majority of the cardiac output (Fig. 14-4). When $P_{alv}$ (and thus $P_{VRG}$) is much less than $P_I$, $P_{MVR}$ is low, and the bloodstream is capable of carrying large amounts of anesthetic away from the alveoli to the tissues. Under these conditions, the rate of uptake of anesthetic from the alveoli into the bloodstream can be greatly modified by cardiopulmonary changes, and $P_{CNS}$ can be greatly affected by changes in ventilation and cardiac output. As each successive tissue group approaches saturation with anesthetic, $P_{MVR}$ approaches $P_I$. When $P_{MVR}$ is nearly equal to $P_I$, the bloodstream cannot remove much anesthetic from the lungs under any circumstances, and changes in ventilation or cardiac output have little effect on $P_{CNS}$.

---

[a] In this hypothetical model, one may correctly note that the *concentration* of Anesthetic B in the CNS *as a whole* will be higher than that of Anesthetic A at any particular time point. One may, therefore, wonder how Anesthetic B can have a slower induction, if anesthesia results when a particular concentration (0.05 M) is reached at the site of action (see Pharmacodynamics, above). At this point, one must recognize that the brain is primarily aqueous, but that anesthetics likely have a *hydrophobic* site of action, and that both Anesthetic A and Anesthetic B must have the same concentration (0.05 M) in the key hydrophobic portions of the brain at the anesthetic partial pressure. However, Anesthetic B, with its larger aqueous solubility (λ(blood/gas)), will partition relatively more than Anesthetic A into the aqueous portions of the brain. To provide the higher aqueous concentrations, many more moles of Anesthetic B than Anesthetic A must be transferred from the lungs.

The overall conclusion still holds if λ(oil/gas) and thus MAC differ for the two hypothetical anesthetics. $P_{alv}$ for a less blood-soluble agent will rise proportionally faster toward its $P_I$ than a more blood-soluble agent, independent of what that $P_I$ is (note that $P_I$ will be larger for the less oil-soluble anesthetic). A larger λ(oil/gas) allows the anesthetic to cause anesthesia at a lower partial pressure, but does not affect the proportional rate at which the partial pressure rises.

Upon commencement of anesthetic administration, the length of time during which there is a significant difference between $P_I$ and $P_{alv}$ increases with $\lambda$(blood/gas). With ventilation-limited anesthetics, such as diethyl ether and halothane, the prolonged time during which $P_{alv}$ lags behind $P_I$ allows cardiopulmonary changes to modulate $P_{alv}$ significantly, potentially leading to unexpected CNS partial pressures. With perfusion-limited anesthetics, such as nitrous oxide, however, the alveolar partial pressure rises so rapidly that $P_{alv}$ is significantly less than $P_I$ for only a short time, minimizing the time during which cardiopulmonary changes could have a significant effect on $P_{CNS}$ (Fig. 14-6).

## Effects of Changes in Ventilation

Hypoventilation decreases the delivery of anesthetic to the alveoli. Meanwhile, removal of anesthetic from the alveoli continues as long as cardiac output is maintained. Consequently, the alveolar partial pressure rises more slowly, and $\tau\{P_{alv} \rightarrow P_I\}$ is prolonged. In other words, *hypoventilation slows induction.* This effect is greater with ventilation-limited than perfusion-limited anesthetics (Fig. 14-9A).

General anesthetics themselves can cause hypoventilation by depressing the medullary respiratory center. In this manner, anesthetic-induced hypoventilation sets up a beneficial negative feedback loop on the depth of anesthesia. Increasing anesthetic depth leads to medullary depression, which, in turn, depresses respiration. The beneficial effect of this physiologic response is that the depressed ventilation slows the rate of rise of the alveolar partial pressure, while perfusion continues to remove anesthetic from the lung at the same rate (Fig. 14-5). Thus, $P_{alv}$ falls, and shortly thereafter, the partial pressure of anesthetic in the medulla falls as well. This decrease in $P_{CNS}$ relieves the respiratory depression. In the extreme example of a full respiratory arrest, there is no ventilation to deliver anesthetic to the alveoli, but cardiac output continues to distribute anesthetic from the alveoli and VRG to the MG and FG. In the case of diethyl ether, the decrease in $P_{CNS}$ can be of a sufficient magnitude that spontaneous ventilation resumes.

Hyperventilation delivers anesthetic more quickly to the alveoli. This decreases the time constant for equilibration of the alveolar with the inspired partial pressure (recall that $\tau\{P_{alv} \rightarrow P_I\} = \text{FRC} / V_{alv}$, in the limiting case). The hyperventilation-induced hypocapnia may concomitantly decrease cerebral blood flow, however, increasing $\tau\{P_{CNS} \rightarrow P_{art}\}$. Thus, while the partial pressure in the alveoli rises faster, the rate of equilibration between the CNS and the alveoli is slower. The net effect depends on which of these two steps is rate limiting. For perfusion-limited anesthetics such as nitrous oxide, the decrease in cerebral blood flow results in a slower induction. For the most soluble ventilation-limited anesthetics such as diethyl ether, the faster delivery of anesthetic to the alveoli hastens induction. For less soluble ventilation-limited anesthetics such as isoflurane, the effects roughly balance and induction is not significantly affected.

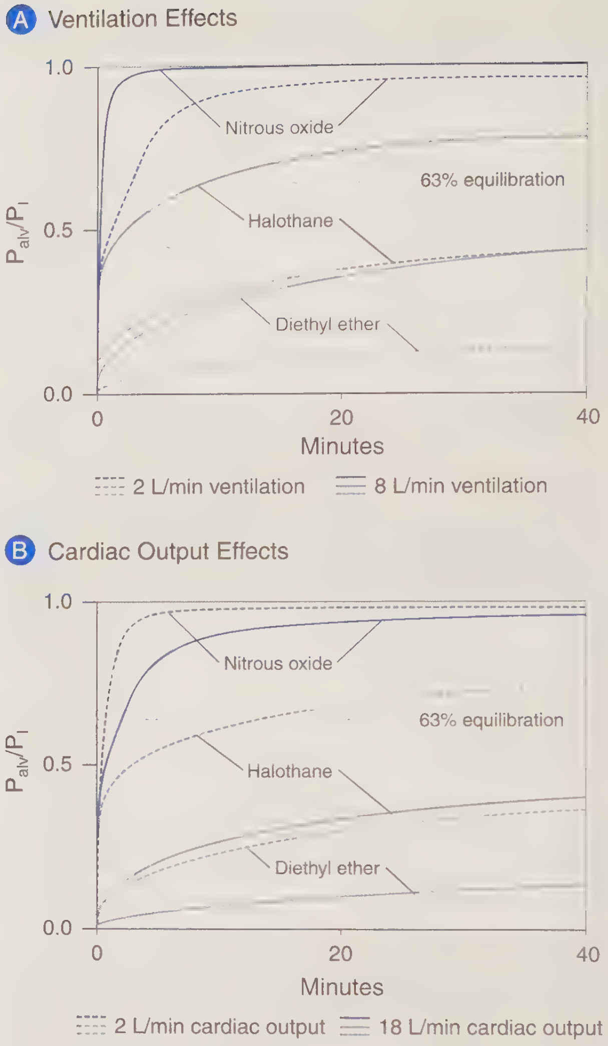


**Figure 14-9. Effects of Changes in Ventilation and Cardiac Output on the Rate at which Alveolar Partial Pressure Rises Toward Inspired Partial Pressure.** The rate of equilibration of the alveolar partial pressure with the inspired partial pressure can be affected by changes in ventilation (A) and cardiac output (B). Increasing ventilation from 2 L/min (dashed lines) to 8 L/min (solid lines) accelerates equilibration. On the other hand, increasing cardiac output from 2 L/min (dashed lines) to 18 L/min (solid lines) slows equilibration. Both effects are much larger for more blood-soluble gases, such as halothane and diethyl ether, which have rather slow induction times. For nitrous oxide, the rate of equilibration is so fast that any changes caused by hyperventilation or decreased cardiac output are small. The dashed horizontal line represents 63% equilibration of $P_{alv}$ with $P_I$; the time required for each curve to cross this line represents $\tau\{P_{alv} \rightarrow P_I\}$.

## Effects of Changes in Cardiac Output

At anesthetic partial pressures higher than those required to depress the respiratory center, cardiac output falls. When cardiac output falls, the bloodstream removes anesthetic from the alveoli at a slower rate. Consequently, the alveolar partial pressure rises faster (Fig. 14-9B). Because the alveolar partial pressure equilibrates relatively quickly with the VRG (even at the lower cardiac output), the partial pressure in the

CNS also rises more rapidly. In other words, *decreased cardiac output speeds induction.* This effect is more marked with ventilation-limited than perfusion-limited anesthetics.

Moreover, cardiac depression by anesthetics sets up a harmful positive feedback loop on the depth of anesthesia. Increasing $P_{CNS}$ depresses cardiac function, which further increases $P_{alv}$, which further increases $P_{CNS}$, which further depresses cardiac function. If cardiac arrest occurs, then positive measures must be taken to restore the circulation while reducing the alveolar partial pressure through controlled breathing with oxygen.

Increased cardiac output increases perfusion to the lungs and accelerates equilibration between the alveoli and the tissues. However, because the increased blood flow to the lungs removes anesthetic from the alveoli at a faster rate, the rate of rise of the alveolar partial pressure is slowed. Thus, *increased cardiac output slows induction.* This effect is greater with ventilation-limited than perfusion-limited agents.

## Effects of Age

Relative to their body weight, young children, such as 7-year-old Matthew, have higher ventilation than do adults. This effect tends to speed induction. However, young children also have relatively higher cardiac output than do adults; this effect tends to slow induction. Although one might expect that these effects would cancel out, there are two additional factors to consider. First, the partial pressure in the mixed venous return rises more quickly in children. This is because, relative to adults, a greater proportion of the blood flow serves the VRG in children, resulting in a higher partial pressure of anesthetic in the mixed venous return early in the course of anesthesia. Second, the increased cardiac output and the lower capacity of the tissues for anesthetic in children relative to adults both accelerate the rate at which the tissues become saturated with anesthetic. Both effects lead to a decreased alveolar-to-venous partial pressure difference, blunting the removal of anesthetic by the pulmonary circulation and moderating the extent to which cardiac output slows the rise in alveolar partial pressure.

Thus, proportional increases in ventilation and cardiac output result in an accelerated rise of alveolar partial pressure and quicker induction in children than in adults (Fig. 14-10). Ventilation-limited anesthetics, which are most affected by cardiopulmonary changes, have a markedly faster induction in children. Therefore, care must be taken to guard against the attainment of unexpectedly high (toxic) levels of anesthetic during anesthesia induction in children.

## Effects of Abnormal States

In hemorrhagic shock, perfusion to the CNS may be maintained in the face of decreased cardiac output and hyperventilation. The decreased cardiac output and hyperventilation both accelerate the rise in alveolar partial pressure of anesthetic. $P_{MVR}$ also rises faster because of the relatively greater perfusion to the VRG, lowering the ability of the pulmonary circulation to remove anesthetic from the alveoli and further accelerating the rise in the alveolar partial pressure. In patients with hemorrhagic shock, the additive combination of these effects can speed induction to a significant degree. In such cases, perfusion-limited anesthetics, which are not greatly affected by cardiopulmonary changes, are preferred over ventilation-limited agents (Fig. 14-9).

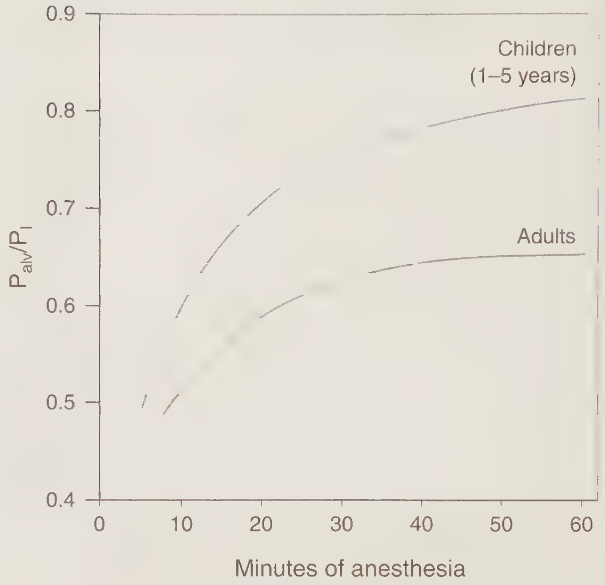


**Figure 14-10. Anesthesia Induction in Children.** Using halothane as an example, the alveolar partial pressure of anesthetic rises more quickly in children than in adults. The faster induction time in children results from a balance between children's increased respiration (favoring faster induction) and increased cardiac output (favoring slower induction); the time-dependent increase in the mixed venous partial pressure of anesthetic limits anesthetic uptake from the lungs, dampening the effect of increased cardiac output on induction time.

In ventilation/perfusion (V/Q) mismatch (e.g., in chronic obstructive pulmonary disease [COPD]), some alveoli are underventilated and overperfused, while others may be adequately ventilated but underperfused. Because less anesthetic is transferred from the underventilated alveoli to the bloodstream, the average partial pressure of anesthetic in the blood leaving the lung is decreased. $P_{CNS}$, thus, equilibrates with a lower than normal arterial partial pressure and may not achieve the level required to induce anesthesia. Therefore, higher inspired partial pressures are necessary to compensate for the effects of V/Q mismatch. This effect is mitigated somewhat with ventilation-limited anesthetics because the partial pressure in the underperfused but overventilated alveoli rises faster than normal. For this reason, perfusion-limited anesthetics are most affected by V/Q mismatch.

Based on the principles and examples discussed above and summarized in Table 14-4, it should be possible to make reasonable predictions about the effect of other changes in cardiopulmonary function on anesthesia induction.

## Control of Induction

An anesthesiologist can decrease induction time by setting the initial $P_I$ higher than the final desired $P_{CNS}$. (This concept is similar to that of a loading dose, which is discussed in Chapter 3.) Because the time constant for equilibration of $P_{CNS}$ with $P_I$ does not depend on the absolute level of $P_I$, administration of anesthetic for a given amount of time always results in the

**TABLE 14-4** Summary of the Effects of Physiologic and Pathophysiologic Variables on Rate of Induction of Anesthesia

| CAUSE FASTER THAN USUAL INDUCTION | CAUSE SLOWER THAN USUAL INDUCTION |
|---|---|
| Hyperventilation (ventilation-limited anesthetics) | Hyperventilation (perfusion-limited anesthetics) |
| Decreased cardiac output | Hypoventilation |
| Young age (i.e., children) | Increased cardiac output |
| Shock | Chronic obstructive pulmonary disease |
| Thyrotoxicosis | Right-to-left shunt |
| Initial $P_I$ higher than final desired $P_{CNS}$ | |

Based on the uptake model for inhaled anesthetics, the effect of changes in physiologic variables on the rate of induction can be predicted. Entities in the column on the left speed induction, while entities on the right slow induction, as discussed in the text. Note that the effect of hyperventilation depends on whether a ventilation-limited or perfusion-limited anesthetic is being administered.

same proportional equilibration of $P_{CNS}$ with $P_I$. Consequently, a given absolute $P_{CNS}$ is reached faster when $P_I$ is higher because that $P_{CNS}$ is a smaller fraction of the higher $P_I$. Dr. Snow took advantage of this concept by starting isoflurane at a $P_I$ of 0.02 atm, even though the MAC of isoflurane is only 0.0114 atm. The anesthesiologist must remember to reduce $P_I$ as $P_{alv}$ approaches the target value, or, as demonstrated by Dr. Snow, $P_{CNS}$ will equilibrate with this higher $P_I$ and cause cardiopulmonary depression (Fig. 14-11).

## RECOVERY

It is desirable that recovery from general anesthesia proceeds quickly, so that patients can maintain their own airways as soon as possible following surgery. In general, the stages of recovery from anesthesia occur in the opposite sequence from those of anesthesia induction, including the unpleasant excitement stage (Fig. 14-1). During recovery, the partial pressure of anesthetic in the mixed venous return ($P_{MVR}$) is

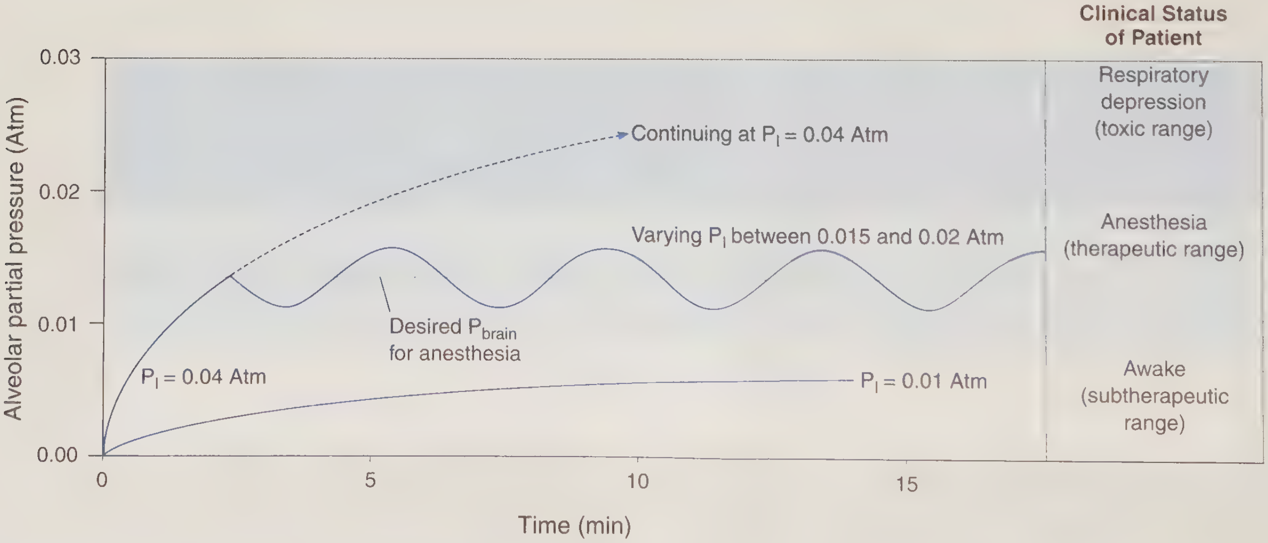


**Figure 14-11.** **Applying Overpressure to Speed Induction.** Using halothane as an example, the anesthesiologist can use an initial $P_I$ greater than the final desired $P_{brain}$ to speed induction. If the desired partial pressure of anesthetic in the brain is about 0.01 atm, then the anesthesiologist could initially administer the inspired anesthetic at a higher partial pressure, for example, 0.04 atm. This method is effective because the time constant for $P_{alv} \rightarrow P_I$ is independent of the absolute value of $P_I$. In other words, if $P_I$ is increased, then the ratio $P_{alv}/P_I$ will increase proportionally at the same rate, resulting in a greater absolute rise in $P_{alv}$ in a given amount of time. The anesthesiologist must be sure to decrease the inspired partial pressure in a timely manner, however, or the desired $P_{brain}$ for anesthesia can be overshot and, instead, partial pressures capable of causing respiratory depression can be reached. On the other hand, if the inspired partial pressure is reduced too rapidly, the patient may awaken as $P_{alv}$ is decreased because of uptake of anesthetic from the alveoli into the bloodstream (not shown).

the weighted average of the partial pressures in the VRG, MG, and FG, with the VRG making the largest contribution (see Fig. 14-4). Ventilation removes anesthetic from the bloodstream into the exhaled air, and therefore, increased ventilation always accelerates recovery. As is the case with induction, recovery from anesthesia with perfusion-limited agents is rapid; whereas, recovery from ventilation-limited agents is more prolonged.

Recovery differs from induction in several important ways, however. First, the anesthesiologist can increase the inspired partial pressure of anesthetic to speed the process of induction, while during recovery, the inspired partial pressure cannot be decreased below zero. Second, during induction, all of the tissue compartments start out at the same partial pressure (zero). In contrast, at the start of recovery, the compartments may have very different partial pressures depending on both the duration of anesthesia and the characteristics of the anesthetic. The VRG quickly equilibrates with the alveolar partial pressure during most surgical procedures, but the MG may or may not equilibrate, and the FG equilibrates so slowly that, in all but the longest procedures, $P_{FG}$ is far from equilibrium. Consequently, during recovery, perfusion **redistributes** anesthetic down its partial pressure gradient from the VRG to the MG and FG as well as to the lung. Because of this redistribution, the initial decrease in alveolar partial pressure during recovery is more rapid than the corresponding increase during induction. This initial decrease in alveolar partial pressure is dominated by the decrease in the VRG partial pressure. When the alveolar pressure falls to the level of the MG, then the decrease in the partial pressure of the MG becomes rate limiting, and likewise subsequently for the FG. If the MG or both the MG and FG are heavily saturated following prolonged administration of anesthetic, then recovery will also be prolonged (Fig. 14-12).

Third, although anesthetic is delivered by one route—ventilation, it can be eliminated by both ventilation and metabolism. In most cases, metabolism is not a significant route of anesthetic elimination. Halothane is an exception because metabolism may account for 20% of its elimination.

Finally, the outflow of high partial pressures of nitrous oxide into the lungs can cause an effect called **diffusion hypoxia.** To understand this, it is helpful first to understand an effect on anesthetic induction called the **concentration effect.** When high partial pressures of nitrous oxide are administered, the rate of anesthetic uptake by the blood may be quite large, on the order of 1 L/min for a 75% nitrous oxide mixture. The absorbed gas is rapidly replaced by inspired gas flowing into the lung, effectively increasing alveolar ventilation by 1 L/min above the normal minute ventilation and, thereby, accelerating induction.

Diffusion hypoxia is conceptually the opposite of the concentration effect. When anesthesia is terminated, nitrous oxide gas diffuses out of the blood into the alveoli at a high rate because of the high partial pressure difference between these two compartments (recall Fick's law). This volume of nitrous oxide displaces up to 1 L/min of air that would otherwise have been inhaled. Thus, the alveolar (and arterial) partial pressure of oxygen falls. The decrease is not significant for a healthy patient, but may be threatening to a compromised patient. To counteract this effect, pure oxygen is routinely administered for a few minutes following anesthesia with nitrous oxide, as Dr. Snow did for Matthew.

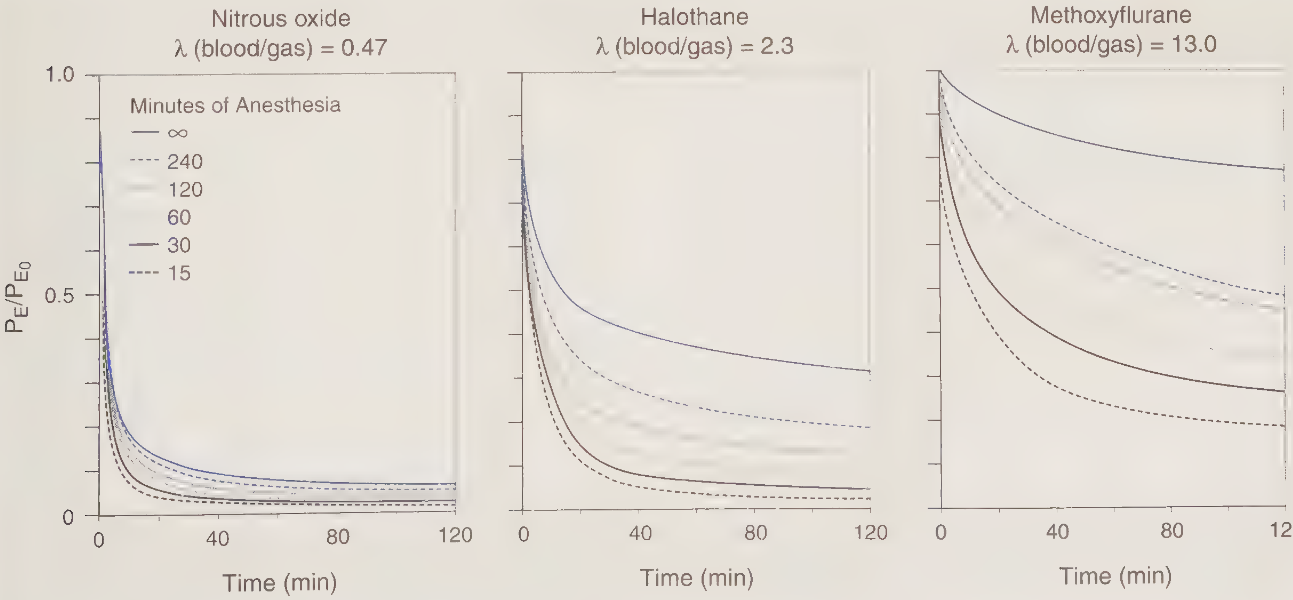


**Figure 14-12. Recovery from Inhaled Anesthetics.** These curves show, as a function of time, the exhaled partial pressure of anesthetic ($P_E$) as a fraction of the exhaled partial pressure at the moment administration of the anesthetic is stopped ($P_{E0}$). The rate of recovery is inversely proportional to the λ(blood/gas) of the anesthetic, because anesthetics with smaller λ(blood/gas) values equilibrate faster between alveolar and inspired partial pressures (the latter being zero after anesthetic administration is stopped). The rate of recovery is also proportional to the duration of anesthesia because the partial pressures of anesthetic in the muscle group and fat group increase with duration. During recovery, anesthetic redistributes from these slowly equilibrating, high-capacity tissues to the vessel rich group, thus slowing the rate of fall of $P_{brain}$.

# PHARMACOLOGY OF GENERAL ANESTHETICS AND ADJUVANTS

## INHALED ANESTHETIC AGENTS

From the preceding analysis, we can distill two physicochemical properties of inhaled anesthetics that predict their behavior. First, the oil/gas partition coefficient predicts potency; *an anesthetic with a larger λ(oil/gas) is more potent and causes anesthesia at lower partial pressures.* Second, the blood/gas partition coefficient predicts the rate of induction; *an anesthetic with a smaller λ(blood/gas) has a shorter induction time.* Typically, there is a trade off between fast induction and high potency. An anesthetic that has a rapid induction, as denoted by a small λ(blood/gas), typically has a low potency, represented by a small λ(oil/gas). Conversely, a very potent anesthetic with a large λ(oil/gas) typically has a large λ(blood/gas) and, thus, a long induction time (see Table 14-1).

**Halothane** has a high λ(oil/gas), providing high potency and, thus, low MAC; however, halothane also has a high λ(blood/gas), causing slow induction and recovery. The nonirritating smell of halothane makes it useful in pediatric anesthesia. One disadvantage of halothane is that toxic metabolites can result in fatal hepatotoxicity. The incidence of this serious adverse effect is approximately 1 in 35,000 in adult populations but much lower in pediatric populations; this is another reason for the continuing role for halothene in pediatric anesthesia. Another rare but potentially lethal side effect, seen most often with halothane but occasionally with the other halogenated anesthetics, is **malignant hyperthermia.** The susceptibility for this adverse reaction is inherited, typically as an autosomal dominant mutation in the sarcoplasmic reticulum $Ca^{2+}$ channel (also known as the ryanodine receptor). In individuals expressing this mutation, halothane causes uncontrolled calcium efflux from the sarcoplasmic reticulum, with subsequent tetany and heat production. Malignant hyperthermia is treated with **dantrolene,** a muscle relaxant that blocks calcium release from the sarcoplasmic reticulum.

**Isoflurane** and **enflurane** are somewhat less potent than halothane (they have a smaller λ(oil/gas)), but they equilibrate faster because they have a smaller λ(blood/gas). Enflurane is metabolically defluorinated to a greater extent than isoflurane, and may, thus, have a greater risk of causing renal toxicity.

Although less potent than isoflurane and enflurane, **diethyl ether** is still quite potent with a rather high λ(oil/gas). However, because of its flammability and very slow induction attributable to its extremely high λ(blood/gas), this agent is no longer in common use in the United States and Europe. In third world countries, however, its low price and simplicity of application ensure its continued use.

**Nitrous oxide** has a very low λ(blood/gas) and, thus, equilibrates extremely rapidly. However, its low λ(oil/gas) results in a very high MAC, close to one atmosphere. Thus, the need to maintain an acceptable partial pressure of oxygen (normally greater than 0.21 atm) prevents the ability to attain full anesthesia using nitrous oxide alone.

**Desflurane** and **sevoflurane** are newer anesthetics that, by design, have low λ(blood/gas) and, therefore, times of equilibration between their alveolar and inspired partial pressures that are nearly as short as nitrous oxide's. Furthermore, they are much more potent than nitrous oxide because their oil/gas partition coefficients are higher. Thus, these agents offer great improvements over earlier agents. However, desflurane is a poor induction agent because its pungency irritates the airway, potentially causing cough or laryngospasm. Sevoflurane is sweet tasting, but can be chemically unstable when exposed to some carbon dioxide adsorbents in anesthetic machinery, degrading to an olefinic compound that is potentially nephrotoxic.

## INTRAVENOUS ANESTHETIC AGENTS

Intravenous anesthetics, such as **barbiturates** (see also Chapter 10), allow for rapid induction. Ultrashort-acting barbiturates, such as **thiopental,** are capable of inducing surgical anesthesia within seconds. All intravenous agents differ significantly from inhaled anesthetics in that they cannot be removed from the body by ventilation. Accordingly, one must take great care during their administration to avoid severe medullary depression that is not easily reversible. The primary method of removal of these agents from the CNS is by redistribution from the VRG to the MG and finally to the FG. Metabolism and/or excretion then slowly decrease the overall body levels of drug (Fig. 14-13).

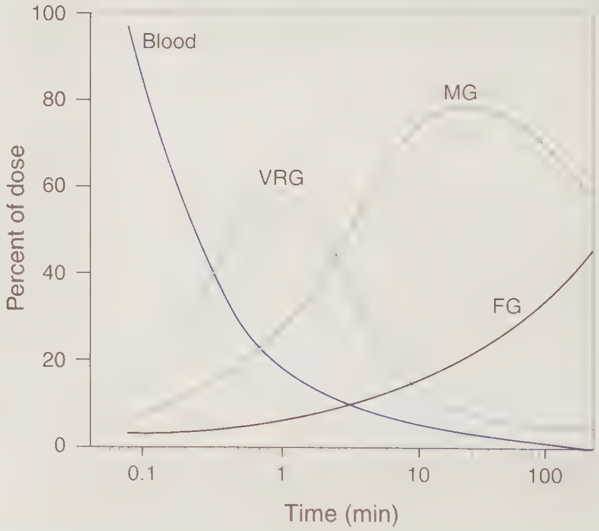


**Figure 14-13. Distribution of a Bolus of Intravenous Anesthetic.** When a bolus of intravenous anesthetic is administered, it is initially transported through the vascular system to the heart and is then distributed to the tissues. The vessel rich group (VRG) receives the highest percentage of the cardiac output; its anesthetic concentration rises rapidly, reaching a peak within 1 minute. Redistribution of anesthetic to the muscle group (MG) then quickly decreases the anesthetic level in the VRG. Because of very low fat group (FG) perfusion, redistribution from the MG to the FG does not occur until much later. Note that rapid redistribution from the VRG to the MG does not occur if the MG has previously approached saturation through prolonged administration of anesthetic (not shown); this can lead to significant toxicity if intravenous barbiturates are administered continuously for long periods of time. New agents, such as propofol, are designed to be eliminated by rapid metabolism and, therefore, can be used safely for longer periods of time.

**Propofol** is an important new intravenous anesthetic in an intralipid formulation. This agent produces anesthesia at a rate similar to the ultrashort-acting barbiturates. Propofol is rapidly metabolized, resulting in a faster recovery than barbiturates. Propofol is used both for induction and for maintenance, especially in short day-surgery procedures where its fast elimination favors prompt recovery and early discharge.

**Etomidate** is an imidazole with kinetics similar to propofol that is used for induction of anesthesia. This agent causes minimal cardiopulmonary depression, perhaps because of its unique lack of effect on the sympathetic nervous system.

Unlike the above agents, **ketamine** produces only dissociative anesthesia, in which the patient seems to be awake but is actually in an analgesic and amnesic state. Ketamine has the unusual property that it increases cardiac output by increasing sympathetic outflow; for this region, it is occasionally useful in emergency trauma situations. However, it can also produce unpleasant hallucinations. This agent is rarely used today.

## ADJUVANT DRUGS

Adjuvant drugs provide additional effects that are desirable during surgery but are not necessarily provided by the general anesthetics. Benzodiazepines (see Chapter 10), such as **diazepam, lorazepam,** and **midazolam** are often given for their anxiolytic and anterograde amnesic properties. These agents are administered 15 to 60 minutes before the induction of anesthesia to calm the patient and obliterate memory of the induction, although they may also be used for intraoperative sedation. If necessary, benzodiazepine effects can be reversed with the antagonist **flumazenil.**

Opioids (see Chapter 15) such as **morphine** and **fentanyl** are used for their ability to produce analgesia. Their action can be reversed pharmacologically; for example, by **naltrexone.** Opioids are poor amnesics, however, and are typically used in combination with a general anesthetic.

The combination of fentanyl and **droperidol** produces both analgesia and amnesia. This combination together with nitrous oxide is called neuroleptanesthesia (the prefix "neurolept" is added because droperidol is a butyrophenone antipsychotic related to haloperidol, see Chapter 11).

Nicotinic acetylcholine receptor blockers, such as the competitive inhibitors **tubocurarine** and **pancuronium** or the depolarizing inhibitor **succinylcholine,** are commonly used to achieve muscle relaxation (see Chapter 7). The effects of the competitive inhibitors can be reversed by using an acetylcholinesterase inhibitor such as **neostigmine.**

## BALANCED ANESTHESIA

No single drug achieves all of the desired goals of anesthesia. Accordingly, in a method termed **balanced anesthesia,** several inhaled and/or intravenous drugs are used in combination to produce the anesthetic state. The effects of simultaneously administered general anesthetics are additive. That is, 0.5 MAC of one inhaled anesthetic in combination with 0.5 MAC of another is equivalent in terms of potency to 1 MAC of either anesthetic as a single agent.

Using a mixture of inhaled anesthetics allows the two goals of potency and rapid recovery to be achieved. For example, although using nitrous oxide alone is generally impractical because the MAC of this gas is higher than atmospheric pressure, nitrous oxide is desirable for its fast induction and recovery characteristics and its high analgesic index. If nitrous oxide is part of the anesthetic mixture, then the nitrous oxide component of the anesthesia can be rapidly removed by ventilation during recovery or in an emergency situation. Matthew was able to awaken quickly from anesthesia because nitrous oxide contributed about half of his anesthetic state. He remained groggy because of the lingering isoflurane. The advantages of using isoflurane in combination with nitrous oxide include the latter agent's low cost and relatively low incidence of side effects (especially hepatic and renal toxicity) as compared to other anesthetics.

Dr. Snow's use of such an intravenous agent as thiopental or propofol in combination with one or more inhaled anesthetic agents has a similar rationale. Short-acting intravenous agents can be used to induce stage III surgical anesthesia quickly, allowing the patient to pass through the undesirable excitement of stage II rapidly. Subsequently, the anesthetic depth can be maintained with inhaled anesthetics that could be removed by ventilation if necessary. Because intravenous agents act additively with inhaled anesthetics, less than 1 MAC of inhaled anesthetic will be required for as long as the intravenous agent is acting. As another example, the use of high concentrations of opioids in cardiac surgery allows the partial pressure of the inhaled anesthetic to be lowered significantly, reducing the risk of cardiovascular and respiratory depression.

Finally, balanced anesthesia is clinically useful because the anesthesiologist has more control if a separate drug is used to mediate each desired effect. For example, if the surgeon requires more muscle relaxation, the anesthesiologist can administer more of a neuromuscular blocking agent without having to increase the depth of anesthesia and potentially cause cardiopulmonary depression. Similarly, a bolus of a short-acting opioid can be administered immediately before a particularly painful surgical maneuver.

## MECHANISM OF ACTION OF GENERAL ANESTHETICS

Despite intensive research, the exact mechanism of anesthetic action remains elusive. The **unitary hypothesis** states that a common mechanism accounts for the action of all anesthetics. Alternatively, each anesthetic, or each class of anesthetic, could have its own mechanism of action. The unitary hypothesis has been traditionally assumed, but recent research has shown that the situation is more complex.

A related question asks whether anesthetics have specific binding sites or act nonspecifically. Traditionally, several clues have suggested the absence of a specific site of action. First, molecules of disparate sizes and structures are capable of causing anesthesia (Fig. 14-14). Assuming the unitary hypothesis, it is difficult to imagine a specific binding site or receptor molecule capable of accommodating such an array of compounds. Second, stereoisomers of volatile anesthetics have generally been found to have equal potency. One criteria for specific binding is that stereoisomers should have different binding constants and, thus, different potencies. Finally, no pharmacologic antagonists of general anesthetics have been

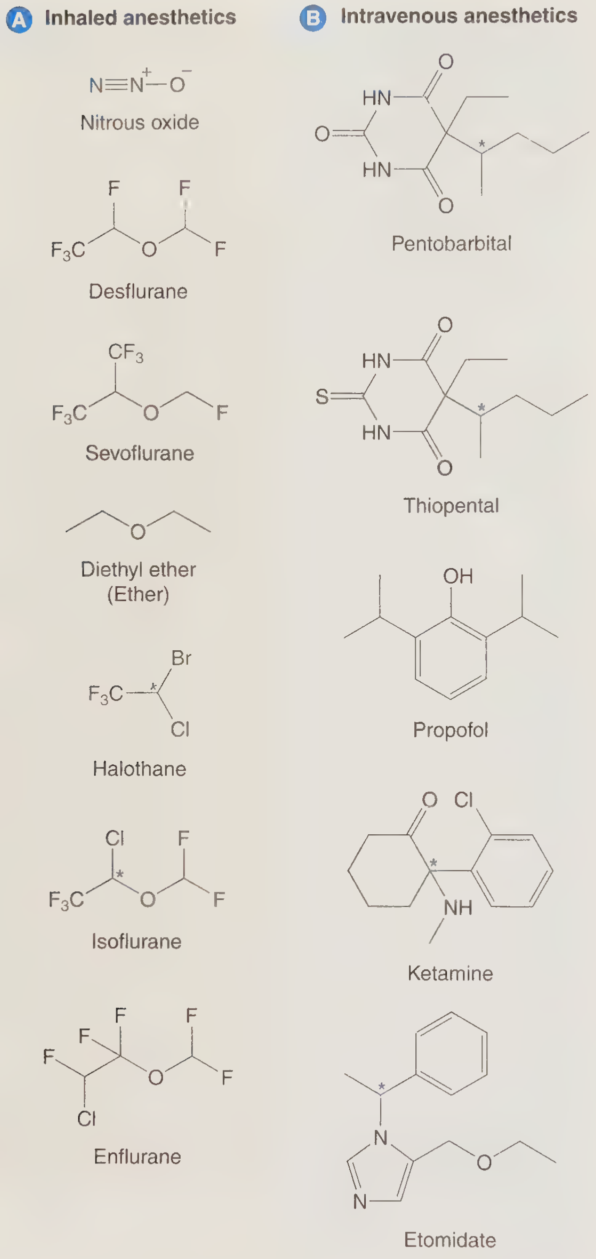


Figure 14-14. Structures of General Anesthetics. **A.** Structures of some inhaled anesthetics. **B.** Structures of some intravenous anesthetics. The extreme variability in the structures of these molecules, all of which are capable of causing general anesthesia, suggests that not all general anesthetics interact with a single receptor site. * Indicates carbons where asymmetry results in enantiomeric structures.

discovered to date, suggesting the lack of a specific site at which an antagonist could compete with a general anesthetic.

## THE MEYER–OVERTON RULE AND THE LIPID SOLUBILITY HYPOTHESIS

Any proposed mechanism of anesthetic action should be consistent with the Meyer–Overton Rule, which suggests a hydrophobic site of action. The **lipid solubility hypothesis,** which postulates that this hydrophobic site of action is the lipid bilayer of a cell, could account for both the Meyer–Overton Rule and the apparent nonspecificity of anesthetic action. According to this hypothesis, general anesthesia results when a sufficient amount of the anesthetic dissolves in the lipid bilayer and a fixed ("anesthetic") concentration is reached. Most lipid theories postulate that the dissolved anesthetic perturbs the properties of the lipid bilayer, which in turn modifies the function of an excitable membrane protein.

Hyperbaric pressure, applied using a nonanesthetic gas (e.g., helium), can reverse anesthesia. This observation supports the lipid perturbation hypotheses, because anesthetics that dissolve in a membrane increase the membrane's volume (by approximately 0.5%) and fluidity. If this volume expansion were the mechanism of general anesthesia, perhaps by affecting excitable transmembrane proteins, then reversal of the volume and fluidity changes with pressure could reverse the anesthesia (this is called the critical volume hypothesis).

The main weakness in the lipid perturbation hypotheses is that no mechanism has been discovered that explains how the small magnitude of the predicted volume or fluidity change would alter the excitability of a membrane. The hypothesis has several more specific weaknesses. First, recent studies have shown that several potent intravenous anesthetics (such as barbiturates, etomidate, and anesthetic steroids) do exhibit significant stereoselectivity. That is, one enantiomer is more potent than the other. Second, many so-called **non-anesthetics** or **nonimmobilizers** are chemically similar to known anesthetics but do not cause anesthesia. For example, straight chain alcohols with more than twelve carbons lack anesthetic activity, even though their $\lambda$(oil/gas) is larger than the shorter alcohols. Other compounds, called transitional anesthetics, have a much higher MAC than that predicted by the Meyer–Overton Rule.

Recently, refinements to the Meyer–Overton Rule have been proposed to account for some of the weaknesses listed above. If interfacial solubilities (i.e., the solubility of a compound at an aqueous–lipid interface) are considered instead of simple lipid solubilities, the Meyer–Overton Rule is much more successful at explaining the activity of transitional and nonanesthetic compounds. This finding is likely to mean that anesthetics act at a hydrophobic–hydrophilic interface. Examples of such an interface would include a water–membrane interface, a protein–membrane interface, or an interface between a hydrophobic protein pocket and the hydrophilic lumen of an ion-conducting pore.

## EFFECTS ON ION CHANNELS

Current research has focused on proteins that may alter neuronal excitability when acted on by anesthetics, either directly or indirectly. Anesthetics affect both axonal conduction and synaptic transmission, but modulation of synaptic transmission occurs at lower anesthetic concentrations and is, therefore, likely to be the pharmacologically relevant action. Accordingly, anesthetics are thought to act on ligand-gated ion channels at lower concentrations than on voltage-gated ion channels. Both presynaptic and postsynaptic modulation occurs, although the postsynaptic actions seem to dominate.

A superfamily of genetically and structurally related ligand-gated channels is sensitive to modulation by anesthetics at clinically relevant concentrations. Members of the superfamily have five homologous subunits, each with four transmembrane regions. The sensitivity of these ligand-gated ion channels to anesthetics can vary with their subunit composition. The superfamily includes excitatory nicotinic acetylcholine and $5HT_3$ receptors, as well as inhibitory $GABA_A$ and glycine receptors (see Fig. 7-2 and Fig. 10-8). Although receptors for glutamate, the major excitatory neurotransmitter in the brain, do not belong to the superfamily, NMDA glutamate receptors are also modulated by some anesthetics (e.g., ketamine and nitrous oxide).

Excitatory receptors (nicotinic acetylcholine, $5HT_3$, and NMDA) are inhibited by anesthetics. The binding of anesthetic to these receptors lowers their maximum activation, without changing the concentration of agonist required to achieve a half-maximal effect ($EC_{50}$) (Fig. 14-15). This action is consistent with noncompetitive inhibition and an allosteric site of action (see also Chapter 2, Pharmacodynamics).

In contrast, inhibitory receptors ($GABA_A$ and glycine) are potentiated by anesthetics. The binding of anesthetic to these receptors decreases the concentration of agonist required to achieve a maximum response, and, thereby, prolongs the synaptic current. The activation curves for these receptors are shifted to the left (lower $EC_{50}$), and the maximal response often increases as well because the anesthetics stabilize the open state of the receptor (Fig. 14-15).

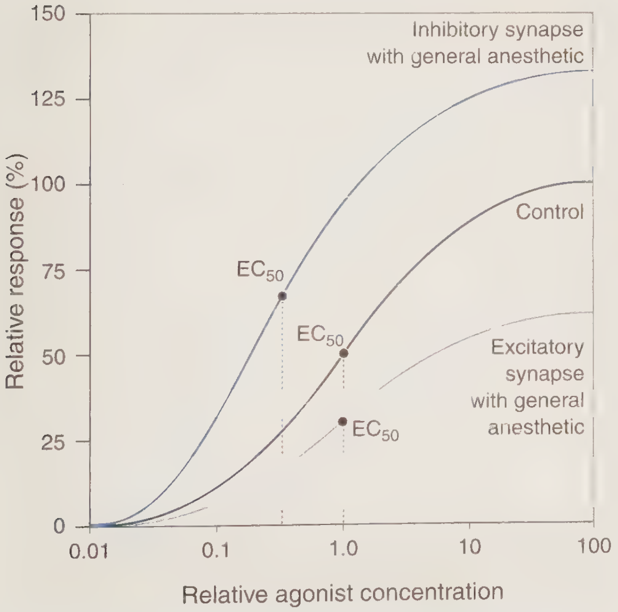


**Figure 14-15. Actions of Anesthetics on Ligand-Gated Ion Channels.** Anesthetics potentiate the action of endogenous agonists at inhibitory receptors, such as $GABA_A$ and glycine receptors, and inhibit the action of endogenous agonists at excitatory receptors, such as nicotinic acetylcholine, $5HT_3$, and NMDA glutamate receptors. At $GABA_A$ receptors, anesthetics both decrease the $EC_{50}$ of GABA (i.e., GABA becomes more potent) and increase the maximum response (i.e., GABA becomes more efficacious). The latter effect is thought to be due to the ability of anesthetics to stabilize the open state of the receptor channel. At excitatory receptors, anesthetics decrease the maximum response while leaving the $EC_{50}$ unchanged; these are the pharmacologic hallmarks of non-competitive inhibition.

Until recently, $GABA_A$ receptors seemed to be the most relevant receptors for general anesthetic action, based on the sensitivity of $GABA_A$ receptors to clinical concentrations of anesthetics and the wide range of agents that act on these receptors. However, it now seems that glycine receptors and some neuronal acetylcholine receptors are equally sensitive to many anesthetics, and that such nonpolar anesthetics as xenon and cyclopropane (both of which have been used in clinical practice) act solely by inhibiting nicotinic acetylcholine and NMDA glutamate receptors.

Direct anesthetic–protein interactions are probably responsible for the effects of anesthetics on ligand-gated ion channels. If anesthetics bind in the pore of excitatory channels, they could directly plug the channel. Alternatively, anesthetics could bind elsewhere and affect the channel's conformation (and, thereby, its equilibrium among the open, closed, and desensitized states). Site-directed mutagenesis, photolabeling, and kinetic studies suggest that inhibition of excitatory acetylcholine receptors probably occurs at a site in the ion channel that is on the central axis of symmetry and in contact with all five subunits. The site of anesthetic binding to the inhibitory $GABA_A$ receptor cannot be in the ion pore because potentiation, not inhibition, is observed at clinical concentrations. Indeed, $GABA_A$ receptors lack a stretch of hydrophobic amino acids that is present in the pore of excitatory receptors. Instead, site-directed mutagenesis suggests an anesthetic binding site located on the outside of the alpha helix that lines the $GABA_A$ ion channel and is bounded by two other transmembrane alpha helices.

Although the emphasis of current research is on protein sites of anesthetic action, no single site yet accounts for the Meyer–Overton Rule or the pharmacology of all general anesthetics. Consequently, adoption of such protein site theories may have to be accompanied by an abandonment of the unitary hypothesis.

## *Conclusion and Future Directions*

Inhaled and intravenous anesthetics provide the components of general anesthesia, including unconsciousness, immobility, and amnesia. The pharmacodynamics of general anesthetics are unique. Anesthetics have steep dose-response curves and small therapeutic indices, and they lack a pharmacologic antagonist. According to the Meyer–Overton Rule, the potency of a general anesthetic can be predicted simply from its oil/gas partition coefficient.

The pharmacokinetics of inhaled anesthetics can be modeled assuming three principal tissue compartments that are perfused in parallel. Equilibration of the partial pressure of anesthetic in the CNS with the inspired partial pressure proceeds in two steps: (1) equilibration between the alveolar partial pressure and the inspired partial pressure; and (2) equilibration between the CNS partial pressure and the alveolar partial pressure. With ventilation-limited anesthetics, which have a large blood/gas partition coefficient, the first of these steps is slow and rate limiting. With perfusion-limited anesthetics, which have a small blood/gas partition coefficient, both steps are rapid, and neither is clearly rate limiting; changes in either can affect induction time. Recovery occurs roughly as the opposite of induction, except that redistribution of the anesthetic from the vessel-rich group to the muscle group and fat group can also occur.

The "ideal" inhaled anesthetic has not yet been found. Future researchers may attempt to identify a nonflammable anesthetic with high $\lambda$(oil/gas), low $\lambda$(blood/gas), a large therapeutic index, good vapor pressure, and few or no significant side effects. Currently, the combined use of adjuvants and balanced anesthesia with multiple inhaled and/or intravenous anesthetics achieves all of the goals of general anesthesia, including fast induction and a state of analgesia, amnesia, and muscle relaxation.

The exact mechanism of action of general anesthetics remains a mystery. Although the site of action was formerly thought to reside in lipid bilayers, direct interactions with several ligand-gated ion channels of the four-transmembrane–helices, Cys-loop superfamily, and of the glutamate receptor family now seem to be more likely. More research is required to elucidate the mechanisms of action of general anesthetics. Once discovered, however, such mechanisms could shed light on such far-reaching issues as the generation of consciousness itself.

## Suggested Readings

Campagna JA, Miller KW, Forman SA. 2003. The mechanisms of volatile anesthetic actions. N Engl J Med 2003;348:2110–2124. (*This article reviews for a broad audience how general anesthetics act.*)

Eger EI. Uptake and distribution. In: Miller RD, ed. Anesthesia. Philadelphia: Churchill Livingstone, 2000:74–95. (*This chapter covers the pharmacokinetics and uptake of inhaled anesthetics.*)

Various authors. 2002. Molecular and basic mechanisms of anaesthesia. In: Hopkins PM, DG Lambert, Urban BW, eds. Brit J Anesth 2002;89:1–183. (*This Postgraduate Educational Issue is a compilation of detailed reviews relating to all major current theories on the mechanism of action of general anesthetics.*)

Wiklund RA, Rosenbaum SH. Anesthesiology. N Engl J Med 1997;337:1132–1141, 1215–1219. (*This two-part review covers many aspects of the modern practice of anesthesiology.*)

Winter PM, Miller JN. Anesthesiology. Sci Am 1985;252:124–131. (*This article is a good account of the clinical approach of the anesthesiologist.*)

# Appendix A:
# Abbreviations and Symbols

$P_I$ = inspired partial pressure

$P_E$ = exhaled partial pressure

$P_{\text{alv}}$ = alveolar partial pressure

$P_{\text{art}}$ = arterial partial pressure

$P_{\text{tissue}}$ = partial pressure in a tissue

$P_{\text{venule}}$ = partial pressure in a venule

$P_{\text{MVR}}$ = mixed venous partial pressure

$P_{\text{solvent}}$ = partial pressure in a solvent

$P_{\text{CNS}}$ = partial pressure in the central nervous system

$P_{\text{VRG}}$ = partial pressure in the vessel rich group

$\lambda(\text{oil/gas})$ = partition coefficient defining solubility of a gas in a lipophilic solvent such as oil

$\lambda(\text{blood/gas})$ = partition coefficient defining solubility of a gas in blood

$\lambda(\text{tissue/gas})$ = partition coefficient defining solubility of a gas in a tissue

$\lambda(\text{tissue/blood})$ = partition coefficient describing ratio of solubility in tissue as compared to solubility in blood

= $\lambda(\text{tissue/gas}) / \lambda(\text{blood/gas})$

$\tau$ = time constant for 63% equilibration

$\tau\{P_{\text{alv}} \rightarrow P_I\}$ = time constant for 63% equilibration of $P_{\text{alv}}$ with $P_I$

$\tau\{P_{\text{tissue}} \rightarrow P_{\text{alv}}\}$ = time constant for 63% equilibration of $P_{\text{tissue}}$ with $P_{\text{alv}}$

[A] = concentration of gas A, in terms of either $L_{\text{gas}}/L_{\text{solvent}}$ or $\text{mol}/L_{\text{solvent}}$

CNS = central nervous system

VRG = vessel rich group (includes CNS, liver, kidney)

MG = muscle group (includes muscle, skin)

FG = fat group (includes adipose tissue)

VPG = vessel poor group (includes bone, cartilage, ligaments, tendons)

FRC = functional residual capacity of lung

$V_{\text{alv}}$ = alveolar ventilation

CO = cardiac output

Q = perfusion rate

$\text{Vol}_{\text{tissue}}$ = volume of tissue

MAC = minimum (or median) alveolar concentration

$P_{50}$ = alveolar partial pressure sufficient for immobility in 50% of patients ≡ MAC

$AP_{50}$ = alveolar partial pressure sufficient to cause analgesia in 50% of patients

$LP_{50}$ = alveolar partial pressure sufficient to cause death in 50% of subjects

$EC_{50}$ = concentration of agonist required to activate 50% of channels

# Appendix B: Equations

### Gas Concentrations

In an ideal gas mixture: $[A]_{\text{mixture}} = n_A / V = P_A / RT$
{in terms of mol/L}

In solution (Henry's Law),
$[A]_{\text{solution}} = P_{\text{solvent}} \times \lambda(\text{solvent/gas})$
{in terms of $L_{\text{gas}}/L_{\text{solvent}}$}

$= P_{\text{solvent}} \times \lambda(\text{solvent/gas}) / 24.5$
{in terms of $\text{mol}/L_{\text{solvent}}$}

{where $n_A$ = moles of gas $A$, $V$ = total volume,
$P_A$ = partial pressure of $A$, $R$ = universal gas constant,
$T$ = temperature in degrees Kelvin}

### Meyer–Overton Rule

$\text{MAC} \approx 1.3 / \lambda(\text{oil/gas})$

### Fick's Law for Diffusion Across a Boundary

Rate of diffusion $= D \times (A / l) \times \Delta P$

{where $D$ = Diffusion constant; $A$ = Surface area;
$l$ = Thickness; $\Delta P$ = Partial pressure difference}

### Alveolar Capillary Rate of Uptake

Rate of uptake $= ([A]_{\text{art}} - [A]_{\text{MVR}}) \times CO$ {in $L_{\text{gas}}/\text{min}$}

Rate of uptake $= \lambda(\text{blood/gas}) \times (P_{\text{art}} - P_{\text{MVR}}) \times CO$

{where $CO$ = cardiac output}

### Equilibration Time Constants (for 63% equilibration)

$\tau$ = Volume Capacity / Flow Rate

$\tau\{P_{\text{tissue}} \rightarrow P_{\text{alv}}\} \approx \tau\{P_{\text{tissue}} \rightarrow P_{\text{art}}\}$
= Volume Capacity of Tissue / Tissue Blood Flow
= $\lambda$(tissue/blood) × Volume of Tissue /
Tissue Blood Flow

$\tau\{P_{\text{brain}} \rightarrow P_{\text{art}}\} = \lambda$(brain/blood) × Volume of brain /
Blood flow to brain

$P_{\text{container}} = P_{\text{flow}}\,[1 - e^{-(t/\tau)}]$

### Volume Capacity

Volume Capacity = $([A]_{\text{compartment}} \times$
Volume of compartment) /
$[A]_{\text{medium}}$ {at equilibrium}
= $\lambda$(compartment/medium)
× Volume of Compartment

### Mixed Venous Partial Pressure

$P_{\text{Venous}} = 0.75\,P_{\text{VRG}} + 0.18\,P_{\text{MG}} + 0.055\,P_{\text{FG}} + 0.015\,P_{\text{VPG}}$

## Drug Summary Table General Anesthetic Pharmacology

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHALED GENERAL ANESTHETICS**<br>*Mechanism—Modulation of ligand-gated ion channels (most likely)* | | | | |
| **Nitrous oxide** | General anesthesia (usually combined with other agents) | Hypoxia during recovery from anesthesia | Expands closed gas spaces (pneumothorax, bowel obstruction) | Fast induction and recovery<br>Relatively weak anesthetic: MAC > 1 atm<br>Analgesia in subhypnotic concentrations<br>Minimal cardiopulmonary depression |
| **Desflurane** | General anesthesia | Cardiovascular and respiratory depression<br>Laryngospasm | All inhaled general anesthetics except nitrous oxide are contraindicated in patients with malignant hyperthermia susceptibility or family history | Fast induction and recovery; pungent, airway irritant |
| **Sevoflurane** | General anesthesia | Cardiovascular and respiratory depression | | Fast induction and recovery; nonirritating vapor |
| **Enflurane** | General anesthesia | Cardiovascular and respiratory depression | | Metabolized to small amounts of inorganic fluoride |
| **Isoflurane** | General anesthesia | Cardiovascular and respiratory depression | | Pungent |
| **Halothane** | General anesthesia | Cardiovascular and respiratory depression<br>Hepatotoxicity<br>Arrhythmias | | Non-irritating vapor |
| **Diethyl ether** | General anesthesia | Cardiovascular and respiratory stimulation<br>Nausea and vomiting | | Slow induction and recovery; pungent, irritating<br>Flammable |
| **INTRAVENOUS GENERAL ANESTHETICS**<br>*Mechanism—Modulation of ligand-gated ion channels (most likely)* | | | | |
| **Thiopental (see Chapter 10)** | Induction of anesthesia | Cardiovascular and respiratory depression | Porphyria | Fast induction and recovery; accumulation after multiple doses |
| **Propofol** | Induction/maintenance of anesthesia, IV sedation | Cardiovascular and respiratory depression | Given in lipid emulsion, possibility of hyperlipidemia and infection | Fast induction and recovery; antiemetic |
| **Etomidate** | Induction of anesthesia | Myoclonus, inhibition of adrenal steroid synthesis | Adrenal insufficiency | Fast induction and recovery |
| **Ketamine** | Dissociative anesthesia/ analgesia | Hallucinations, cardiovascular stimulation, myoclonus, salivation | Increased intracranial pressure<br>Hypertension, cardiac disease, congestive heart failure | Minimal cardiopulmonary depression<br>NMDA antagonist<br>Causes sympathetic activation (see contraindications) |

*(Continued)*

## Drug Summary Table General Anesthetic Pharmacology *(Continued)*

| Drug | Clinical Uses | Side Effects / Toxicity | Interactions / Contraindications | Notes |
|---|---|---|---|---|
| **BENZODIAZEPINES**<br>*Mechanism—Potentiation of $GABA_A$ receptors* | | | | |
| **Diazepam**<br>**Lorazepam**<br>**Midazolam** | All benzodiazepines can be used for sedation, hypnosis, amnesia, anxiolysis, anticonvulsant effect | All benzodiazepines can potentiate other CNS depressants | Benzodiazepines are contraindicated in narrow angle glaucoma and pregnancy<br>Benzodiazepines can be reversed by flumazenil | Diazepam is long-acting<br>Lorazepam is intermediate-acting<br>Midazolam is short-acting |
| **OPIOIDS** (see Chapter 15)<br>*Mechanism—Opioid receptor agonists* | | | | |
| **Morphine**<br>**Meperidine**<br>**Fentanyl**<br>**Remifentanil** | All opioids can be used in adjuvant analgesia | All opioids can cause respiratory depression, bradycardia, miosis | Benzodiazepines greatly increase sedative and respiratory effects<br>Opioids can be reversed by naloxone or naltrexone | Opioids do not produce hypnosis or amnesia |
| **NEUROMUSCULAR BLOCKERS** (see Chapter 7)<br>*Mechanism—Depolarizing or non-depolarizing inhibition of neuromuscular nicotinic acetylcholine receptors* | | | | |
| **Tubocurarine**<br>**Pancuronium** | Long duration adjuvant paralysis | Hypotension, tachycardia | Renal dysfunction<br>Myasthenia gravis<br>Aminoglycosides | Non-depolarizing blockade, reversible with inhibitors of acetylcholinesterase, cross-reacts with muscarinic receptors, mainly renal elimination |
| **Vecuronium**<br>**Cis-atracurium** | Intermediate duration adjuvant paralysis | | Myasthenia gravis<br>Aminoglycosides | Non-depolarizing blockade, reversible with inhibitors of acetylcholinesterase, cis-atracurium's elimination is independent of organ function |
| **Mivacurium** | Short duration adjuvant paralysis | | Myasthenia gravis<br>Aminoglycosides | Non-depolarizing blockade, reversible with inhibitors of acetylcholinesterase, releases histamine, prolonged action in patients with pseudocholinesterase deficiency |
| **Succinylcholine** | Very short duration adjuvant paralysis | Fasciculations, muscle pain, hyperkalemia; raised intracranial, intraocular, and intragastric pressures | Malignant hyperthermia susceptibility, Duchenne's or myotonic dystrophy, burn patients, upper motor neuron injury | Depolarizing blockade, not reversible, prolonged action in patients with pseudocholinesterase deficiency, cross-reacts with muscarinic receptors |

# 15

# Pharmacology of Analgesia

Robert S. Griffin and Clifford J. Woolf

**Introduction**
**Case**
**Physiology**
    Sensory Transduction: Excitation of Primary Afferent Neurons
    Conduction from the Periphery to the Spinal Cord
    Transmission in the Dorsal Horn of the Spinal Cord
    Descending and Local Inhibitory Regulation in the Spinal Cord
**Pathophysiology**
    Clinical Pain
    Peripheral Sensitization
    Central Sensitization
    Neuropathic Pain
    Migraine
**Pharmacologic Classes and Agents**
    Opioid Receptor Agonists
        *Mechanism of Action and Major Side Effects*
        *Morphine, Codeine, and Derivatives*
        *Synthetic Agonists*
        *Partial and Mixed Agonists*
        *Opioid Receptor Antagonists*
    Nonsteroidal Anti-Inflammatory Drugs and Nonopioid Analgesics
        *General Features*
        *Specific Agents*
    Antidepressants
    Anticonvulsants and Antiarrhythmics
    NMDA Receptor Antagonists
    Adrenergic Agonists
    Migraine Therapy
**Conclusion and Future Directions**
**Acknowledgments**
**Suggested Readings**

## INTRODUCTION

Everyone has experienced mild pain in response to an intense or noxious stimulus. This pain helps us to avoid potential damage by acting as an early warning signal. In this respect, pain can be an early diagnostic symptom for the onset of many illnesses. Pain can also be severe and incapacitating, as after an injury, during recovery from surgery, or in association with medical conditions such as rheumatoid arthritis. Under these circumstances, noxious stimuli can elicit severe pain because of increases in the excitability of the somatosensory system, and stimuli that would not normally cause pain can become painful. In addition, nerve injury produced by disease or trauma, as in amputation, HIV infection, varicella zoster (VZV) infection, and diabetes mellitus, can produce pain that persists long after the initiating cause has disappeared. In these conditions, pathologic alterations in the structure and function of the nervous system can lead to the experience of severe and intractable pain. For such patients, the pain is the pathology rather than a physiologic defense mechanism.

Each of these categories of pain—physiologic, inflammatory, and neuropathic—is produced by different mechanisms. Ideally, treatment should be targeted at these specific mechanisms. A number of pharmacologic agents are currently available to relieve pain. These drugs have mechanisms of action that may interfere with the response of primary sensory neurons to somatic or visceral sensory stimuli, with the relaying of information to the brain, and with the perceptual response to a painful stimulus. The following discussion of pain and analgesic pharmacology begins by describing the mechanisms by which noxious stimuli can lead to the perception of pain. The chapter continues by considering the processes responsible for the heightened pain sensitivity that can occur in response to inflammation and to lesions of the nervous system. The chapter concludes by describing the mechanisms of action of the major drug classes used for clinical pain relief.

## Case

JD, a 15-year-old boy, receives severe burns while escaping from a building fire. The burns are extensive, including first and second degree burns covering much of his body and a local, full-thickness burn on his right forearm. He reaches the emergency room in severe pain, and is treated with IV morphine in increasing quantities until he reports that the pain has subsided. This dose of morphine is then maintained. The next day, he receives a skin graft covering the region of his full-thickness burn. During the operation, an anesthesiologist provides a continuous IV infusion of remifentanil, with a bolus dose of morphine added 15 minutes before the end of the operation. At the end of the operation, and for 4 days thereafter, JD receives IV morphine through a patient-controlled analgesia device. As the burns heal, the morphine dose is tapered and eventually replaced with an oral codeine/acetaminophen combination tablet. Three months later, JD reports severe loss of sensation to touch in the area of the skin graft. He also describes a persistent tingling sensation in this area, with occasional bursts of sharp, knife-like pain. After referral to a pain clinic, JD is prescribed oral gabapentin, which partially reduces his symptoms. However, he reports to the pain clinic again 2 months later, still in severe pain. At this time, amitriptyline is added to the gabapentin, and the pain is further relieved. Three years later, JD's lingering pain has resolved and no longer requires medication, but the lack of forearm sensation persists.

## QUESTIONS

1. What mechanisms produced and sustained the pain that lasted from JD's exposure to the fire until his initial treatment?
2. What was the rationale for the sequence of medications used during the skin graft operation?
3. Why was morphine tapered gradually and replaced with a combination codeine/acetaminophen tablet?
4. Explain the mechanisms that could produce spontaneous pain in the region of the full-thickness burn months to years after healing of the skin graft, and the rationale for using gabapentin to treat JD's chronic pain.

## PHYSIOLOGY

**Pain** is the end perceptual result of neural processing of particular sensory information that is usually initiated in the periphery and transferred under multiple controls through sensory relays in the CNS to the cortex. This system can be usefully analyzed in terms of the sites of action at which drugs intervene to produce analgesia. First, transduction of intense external stimuli depolarizes the peripheral terminals of "high-threshold" primary sensory neurons. (The primary sensory neurons are termed "high-threshold" because they require a strong, potentially tissue-damaging stimulus to depolarize their terminals.) The resulting action potentials are conducted to the central nervous system by the axons of the primary afferent sensory neurons, running first in peripheral nerves and then in dorsal roots, which then synapse on neurons in the dorsal horn of the spinal cord. The secondary projection neurons transmit information to the brainstem and thalamus, which then relay the signal to the cortex, hypothalamus, and limbic system. Transmission is modulated at all levels of the nervous system by remote and local-circuit inhibitory and excitatory interneurons (Fig. 15-1).

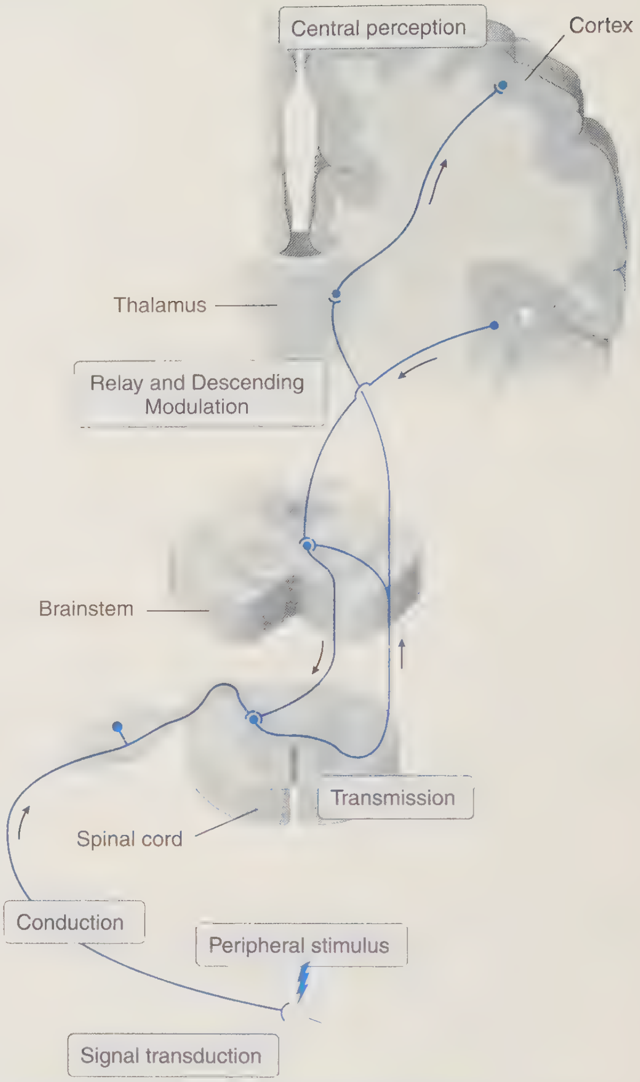


**Figure 15-1. Overview of the Nociceptive Circuit.** Activation of the peripheral terminal by a noxious stimulus leads to the generation of action potentials, which are conducted to the dorsal horn of the spinal cord. Neurotransmission in the dorsal horn relays the signal to CNS neurons, which send the signal to the brain. This circuit is also subject to descending modulatory control.

## SENSORY TRANSDUCTION: EXCITATION OF PRIMARY AFFERENT NEURONS

The peripheral terminals of primary afferent somatic and visceral sensory fibers respond to thermal, mechanical, and chemical stimuli (Fig. 15-2). Highly specialized ion channels undergo conformational changes in response to one or more of these stimuli, and, thereby, mediate the depolarization (generator potential) necessary to initiate an action potential. The firing frequency and duration of the action potentials in the activated fiber then transfer to the CNS information about the onset, intensity, and location of the stimulus.

Thermal pain sensitivity depends on distinct populations of primary sensory neurons: some become active at cold temperatures (<10°C), whereas others respond to heat. Heat pain sensing neurons produce action potentials at temperatures above 42°C. Responses to noxious heat involve thermosensitive cation channels, particularly **TRPV1,** which is a member of the

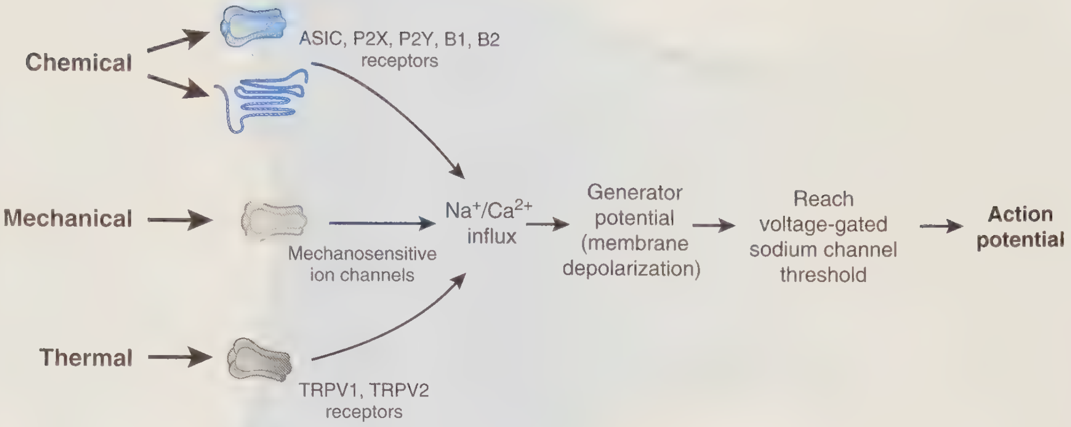

Chemical
ASIC, P2X, P2Y, B1, B2 receptors
Mechanical
Mechanosensitive ion channels
Thermal
TRPV1, TRPV2 receptors
Na⁺/Ca²⁺ influx
Generator potential (membrane depolarization)
Reach voltage-gated sodium channel threshold
Action potential

**Figure 15-2.** **Peripheral Transduction.** A thermal, chemical, or mechanical sensory event activates a specific peripheral receptor, leading to ion influx and depolarization of the peripheral terminal. Thermal stimuli activate the transient receptor potential (TRP), vanilloid receptor 1 (TRPV1), or the TRP vanilloid-receptor like protein 1 (TRPV2), both of which are heat-sensitive cation channels. Chemical stimuli can activate acid sensitive ion channels (ASIC), ATP-sensitive P2X or P2Y channels, or kinin-sensitive B1 or B2 receptors. Mechanical stimuli can also lead to ion influx and depolarization, but the molecular identity of the relevant channels is not certain. In each case, the generator potential induced by the nociceptive signal leads to action potential production if the threshold for activation of the voltage-sensitive sodium channel is reached.

TRP transient receptor potential family of ion channels. This channel becomes active in response to low extracellular pH, vanilloid chemical ligands such as capsaicin (the pungent ingredient in chili peppers), or heat in excess of 42°C. In addition to TRPV1, a second vanilloid receptor, **TRPV2,** is activated only above 50°C. TRPV heat-sensitive ion channels represent targets for the development of new drugs to interfere with peripheral heat sensation. In JD's case, the initial experience of pain was mediated by heat-activation of thermosensitive high threshold peripheral neurons expressing TRPV1 or TRPV2.

Similarly, a specific subpopulation of primary afferent terminals (high threshold mechanoreceptors) is excited by relatively intense mechanical stimuli, such as a pinch or a pin-prick. This excitation is likely to be mediated by a different family of cation channels, the degenerin epithelial sodium channels (ENaC).

The peripheral terminals of **nociceptive neurons** (i.e., neurons that respond to noxious stimuli) can respond not only to such physical energy as thermal and mechanical stimuli, but also to multiple chemical signals. Some chemical agents directly excite peripheral terminals (**chemical activators**), whereas others modulate the excitation thresholds of peripheral terminals (**sensitizing agents**). Most known chemical ligands that evoke a somatosensory response are associated with cell injury or inflammation. These chemical ligands include protons, potassium ions, ATP, amines, cytokines, and bradykinin. For example, cardiac angina is a nociceptive event that involves activation of visceral chemotransducers in nociceptor neurons innervating the heart. These chemotransducers are activated by protons that are released by inadequately perfused myocardial tissue.

Three types of chemical stimuli can excite nociceptive neurons (Table 15-1). First, low extracellular pH, which occurs in ischemia and inflammation, produces depolarizing cation influx through both TRPV1 and **acid sensitive ion channels (ASICs).** The acid sensing sodium ion channels belong to a single superfamily, **degenerin/ENaC.** Second, elevated extracellular ATP concentration also signals cell injury, because cell rupture releases millimolar concentrations of ATP into the extracellular space (where the ATP concentra-

**TABLE 15-1** Types of Nociceptors

| NOCICEPTIVE STIMULUS | NOCICEPTOR | TYPE OF RECEPTOR |
|---|---|---|
| Low pH ($H^+$) | ASIC | pH gated ion channel |
| ATP | P2X | Ligand gated ion channel |
| | P2Y | G protein-coupled receptor |
| Kinin peptides | B1 | $G_q$ protein-coupled receptor |
| | B2 | $G_q$ protein-coupled receptor |

tion is normally very low). There are two major classes of ATP receptor, the **P2X** ligand-gated channels and the **P2Y** G protein-coupled ATP receptors.

**Kinins** are a third chemical stimulus effective in exciting the peripheral terminals of sensory neurons. Kinin peptides are produced from the kininogens by serine protease kallikreins; this process occurs in the setting of inflammation and tissue damage. Kinins act by stimulating **bradykinin B1** and **B2** receptors. The B2 receptor is constitutively expressed throughout the nervous system, while expression of the B1 receptor is induced in response to the inflammatory cytokines IL-1β, TNF-α, IL-2, and IL-9, and to bacterial lipopolysaccharide. Both kinin receptors are G protein-coupled and increase intracellular calcium by production of inositol 1,4,5-trisphosphate. Activation of the B2 receptor also leads to the formation of prostaglandins $E_2$ and $I_2$. In the introductory case, as the heat sensation was followed by burn injury, these chemical mediators further contributed to JD's pain.

Each of these chemosensitive receptors represents a potential target for future drug development. Antagonists of the ASICs or P2X/P2Y channels and antagonists of the B1 or B2 receptors could be useful for reducing acute pain caused by tissue damage and inflammation. In addition, such antagonists could have a role in preventing peripheral sensitization during inflammatory pain (see below).

## CONDUCTION FROM THE PERIPHERY TO THE SPINAL CORD

The axons of primary afferent neurons conduct information from the peripheral terminal to the central nervous system. These neurons can be classified into three major groups according to their conduction velocity and caliber; these groups also have distinct stimulus sensitivities and distinct central termination patterns. The first group (**Aβ**) consists of rapidly conducting fibers that respond with a low stimulus threshold to mechanical stimuli. Aβ fibers synapse on central nervous system neurons located in the dorsal horn of the spinal cord and in dorsal column nuclei of the brainstem. The second population (**Aδ**) includes fibers that conduct with intermediate velocity and respond to cold, heat, and high-intensity mechanical stimuli. The third group (**C fibers**) conduct slowly, synapse in the spinal cord, and typically respond multimodally; they are capable of producing action potentials in response to heat, warmth, intense mechanical stimuli, or chemical irritants (polymodal nociceptors). Some C-fiber afferents (referred to as silent, or sleeping fibers) cannot be activated normally, but become responsive during inflammation. Aδ and C fibers terminate in the most superficial laminae of the dorsal horn (lamina I and II).

For conduction to occur, voltage-gated sodium ion channels must convert depolarization of the peripheral terminal into an action potential. Six types of voltage-gated sodium channel are expressed in primary afferent neurons, of which four, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, and $Na_x$, are expressed uniquely in primary afferents. $Na_v1.8$ and $Na_v1.9$ are selectively expressed in small caliber neurons, most of which respond to high-threshold peripheral stimuli ("pain" fibers). These two channel types also have higher activation thresholds and inactivate more slowly than other neuronal voltage-gated sodium channels. Because of their specific expression pattern in pain fibers, these two voltage-gated sodium channels represent pharmacologic targets of particular interest. Selective blockade of these sensory neuron-specific channels may inhibit pain induced in the periphery without blocking tactile sensibility or somatic or autonomic motor function and without acting on CNS or cardiovascular sodium channels. Currently, the use of sodium channel blocking drugs, such as local anesthetics (see Chapter 9, Local Anesthetic Pharmacology) and anticonvulsants (see Chapter 13, Pharmacology of Abnormal Electrical Neurotransmission in the Central Nervous System), are limited by the side effects associated with nonselective blockade of voltage-gated sodium channels.

## TRANSMISSION IN THE DORSAL HORN OF THE SPINAL CORD

Action potentials generated in primary afferents induce neurotransmitter release upon reaching the central axon terminals in the dorsal horn of the spinal cord. **N-type voltage-gated calcium channels** have a substantial role in controlling this neurotransmitter release from synaptic vesicles. A naturally occurring snail poison, omega-conotoxin, acts as a selective N-type calcium channel blocker; a synthetic mimic of this peptide is currently being tested for use in specific pain conditions. Such calcium channel blockers would, however, be expected to alter the function of sympathetic neurons (producing hypotension) and many central neurons (affecting cognitive function). Thus, the use of these agents will likely be limited to intrathecal administration, in an effort to localize their effects to the spinal cord.

Synaptic transmission in the dorsal horn between C-fiber primary afferents and secondary projection neurons has fast and slow components (Fig. 15-3). Glutamate mediates fast excitatory transmission between primary and secondary sensory neurons. **Neuropeptides,** such as **tachykinins** and **calcitonin gene-related peptide (CGRP),** as well as other **synaptic neuromodulators,** including such **neurotrophins** as **brain-derived neurotrophic factor (BDNF),** are co-released with glutamate and produce slower synaptic effects by acting on metabotropic G-protein coupled receptors and receptor tyrosine kinases. The presence of these co-released peptides allows considerable use-dependent functional plasticity in the control of pain transmission. The physiologic function of the neuropeptides in synaptic transmission most likely involves signaling responses to stimuli of particularly high intensity, because release of neuropeptide-containing synaptic vesicles requires higher-frequency and longer-lasting action potential trains than release of glutamate-containing vesicles.

## DESCENDING AND LOCAL INHIBITORY REGULATION IN THE SPINAL CORD

Synaptic transmission in the spinal cord is regulated by the actions of both local inhibitory interneurons and projections that descend from the brainstem to the dorsal horn. Because these systems limit transfer of incoming sensory information to the brain, they represent an important site for pharmacologic intervention. The major inhibitory neurotransmitters in the dorsal horn of the spinal cord are **opioid** peptides, **norepinephrine, glycine,** and **GABA** (Fig. 15-4).

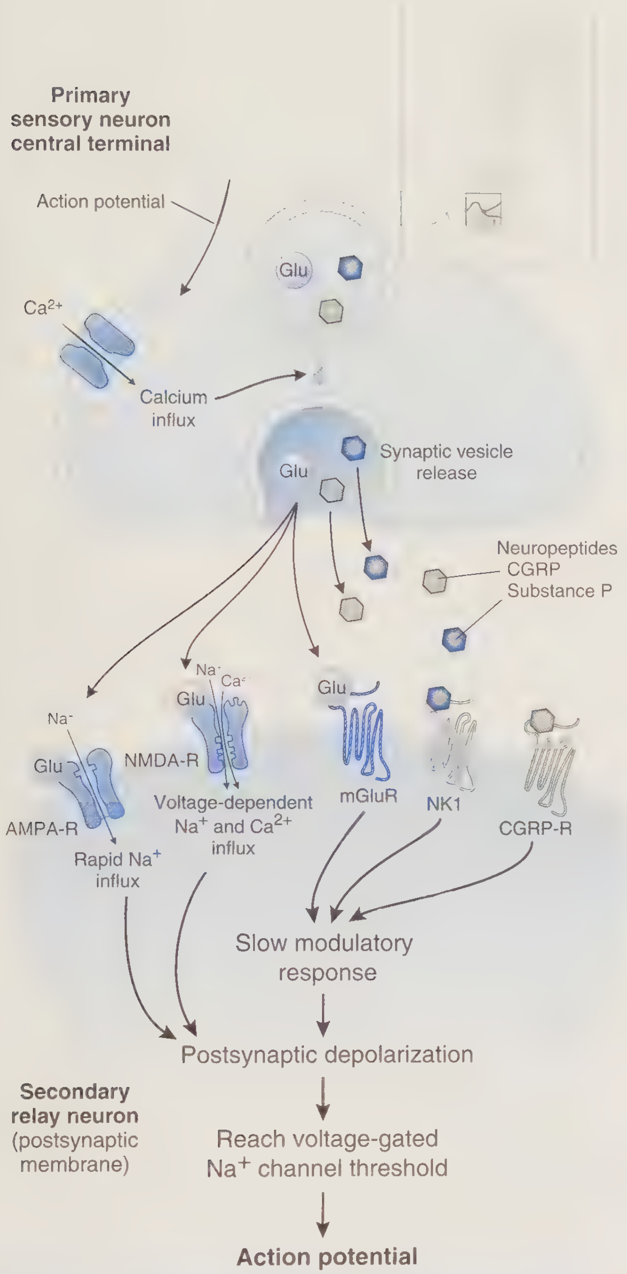

Primary sensory neuron central terminal
Action potential
$Ca^{2+}$
Glu
Calcium influx
Glu
Synaptic vesicle release
Neuropeptides CGRP Substance P
$Na^+$
$Ca^{2+}$
Glu
Glu
$Na^+$
Glu
NMDA-R
AMPA-R
Voltage-dependent $Na^+$ and $Ca^{2+}$ influx
mGluR
NK1
CGRP-R
Rapid $Na^+$ influx
Slow modulatory response
Postsynaptic depolarization
Secondary relay neuron (postsynaptic membrane)
Reach voltage-gated $Na^+$ channel threshold
Action potential

**Figure 15-3. Neurotransmission in the Spinal Cord Dorsal Horn.** An incoming action potential from the periphery activates presynaptic voltage-sensitive calcium channels, leading to calcium influx and subsequent synaptic vesicle release. The released neurotransmitters (i.e., glutamate and neuropeptides, such as calcitonin gene-related peptide [CGRP] and substance P) then act on postsynaptic receptors. Stimulation of ionotropic glutamate receptors leads to fast postsynaptic depolarization, while activation of other modulatory receptors mediates slower depolarization. Postsynaptic depolarization, if sufficient, leads to action potential production (signal generation) in the secondary relay neuron.

The opioid peptides inhibit synaptic transmission, and are released at several CNS sites in response to noxious stimuli. All endogenous opioid peptides, which include **β-endorphin,** the **enkephalins,** and the **dynorphins,** share the N-terminal sequence Tyr-Gly-Gly-Phe-Met/Leu. The opioids are proteolytically released from the larger precursor proteins proopiomelanocortin, proenkephalin, and prodynorphin. Opioid receptors fall into three classes, designated **μ, δ,** and **κ,** all of which are seven transmembrane G protein-coupled receptors. The μ-opioid receptors are critical for morphine-induced analgesia. This conclusion is based on the observation that the μ-opioid receptor knockout mouse exhibits neither analgesia nor side effects in response to **morphine** administration. The endogenous opioid peptides are receptor-selective; the dynorphins act primarily on κ receptors, while both enkephalins and β-endorphin act on the μ and δ receptors. The known effects of opioid receptor signaling include reduced presynaptic calcium conductance, enhanced postsynaptic potassium conductance, and reduced adenylyl cyclase activity. The first function impedes presynaptic neurotransmitter release; the second reduces postsynaptic neuronal responses to excitatory neurotransmitters; the physiologic role of the last remains unknown.

Opioids produce analgesia because of their action in the brain, brainstem, spinal cord, and peripheral terminals of primary afferent neurons. In the brain, opioids alter mood and the reaction to pain. In the brainstem, opioids increase the activity of cells that provide descending inhibitory innervation to the spinal cord. Spinal opioids inhibit synaptic vesicle release from primary afferents and hyperpolarize postsynaptic neurons. There is also some evidence that peripheral opioid receptor activation reduces the activation of primary afferents. Action of opioids at these serially located sites is thought to have a synergistic effect to inhibit information flow from the periphery to the brain.

Norepinephrine is released by projections that descend from the brainstem to the spinal cord. The $\alpha_2$-adrenergic receptor, a seven transmembrane G protein-coupled receptor (see Chapter 8, Adrenergic Pharmacology), is the primary receptor for norepinephrine in the spinal cord. As with opioid receptor activation, $\alpha_2$-adrenergic receptor activation opens postsynaptic potassium channels, inhibits presynaptic voltage-gated calcium channels, and inhibits adenylyl cyclase. Because $\alpha_2$-adrenergic receptors are expressed both presynaptically and postsynaptically, spinal norepinephrine release can both reduce presynaptic vesicle release and decrease postsynaptic excitation. The $\alpha_2$-adrenergic receptor agonist **clonidine** is sometimes used to treat pain, although the applications are limited by such side effects as sedation and postural hypotension. Serotonin is also released in the spinal cord by projections that descend from the brainstem. This neurotransmitter seems to act on several receptor subtypes that mediate both excitatory and inhibitory effects on nociception. Given this complexity, the mechanism of the analgesic effect is not fully understood. Selective serotonin reuptake inhibitors have been tested in the treatment of pain but have had little beneficial effect.

Other compounds may also have regulatory roles in the spinal cord. The **cannabinoid receptors** and the endogenous cannabinoids have recently become a focus for research on the regulation of pain. There are two cannabinoid receptors, both of which are G protein-coupled: CB1, expressed in the brain, spinal cord, and sensory neurons, and CB2, expressed in non-neural tissues, largely in immune cells. Thus far, two endogenous cannabinoids, **anandamide** and **2-arachidonylglycerol,** have been identified. Anecdotal evidence suggests that marijuana has an analgesic effect in patients with AIDS neuropathy or multiple sclerosis. However, this observation requires confirmation in controlled clinical trials, ideally using selective CB receptor agonists.

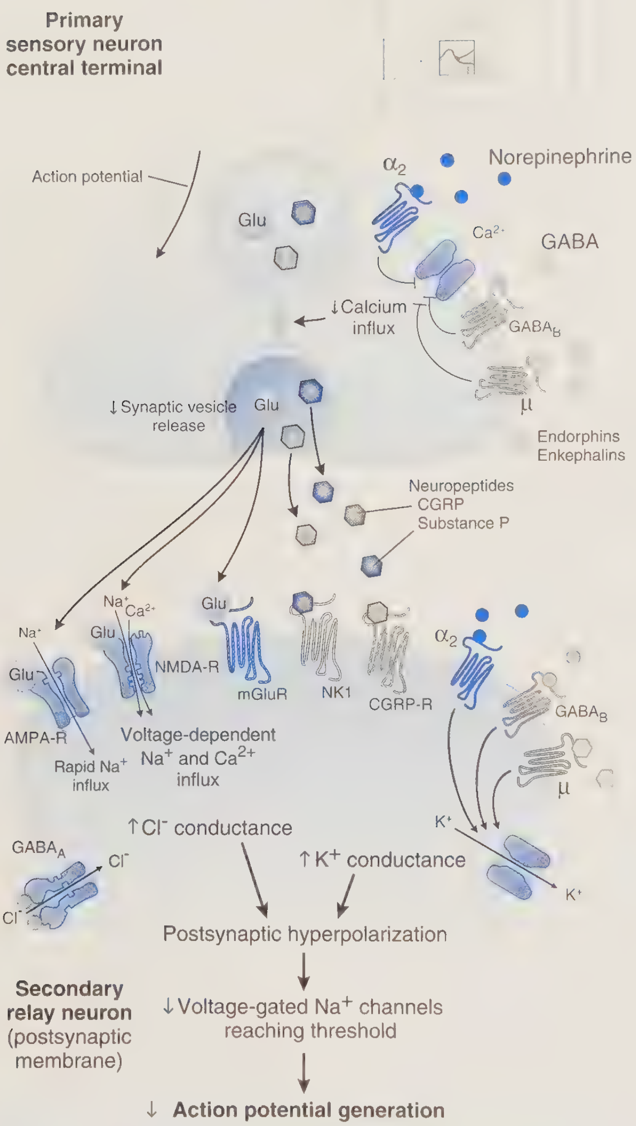

Primary sensory neuron central terminal
Action potential
Glu
$\alpha_2$
Norepinephrine
$Ca^{2+}$
GABA
↓Calcium influx
$GABA_B$
↓Synaptic vesicle release
Glu
μ
Endorphins
Enkephalins
Neuropeptides
CGRP
Substance P
$Na^+$
$Ca^{2+}$
Glu
$Na^+$
Glu
Glu
NMDA-R
mGluR
NK1
CGRP-R
$\alpha_2$
$GABA_B$
AMPA-R
Voltage-dependent $Na^+$ and $Ca^{2+}$ influx
Rapid $Na^+$ influx
μ
↑$Cl^-$ conductance
$K^+$
$GABA_A$
$Cl^-$
↑$K^+$ conductance
$Cl^-$
$K^+$
Postsynaptic hyperpolarization
Secondary relay neuron (postsynaptic membrane)
↓Voltage-gated $Na^+$ channels reaching threshold
↓ Action potential generation

**Figure 15-4. Inhibitory Regulation of Neurotransmission.** Norepinephrine, GABA, and opioids, released by descending and/or local-circuit inhibitory neurons, act both presynaptically and postsynaptically to inhibit neurotransmission. Presynaptic inhibition is mediated through reduced activity of voltage-sensitive calcium channels, whereas postsynaptic inhibition is mediated primarily by enhanced chloride influx and potassium efflux.

## PATHOPHYSIOLOGY

The pain processing circuit described above is responsible for producing acute **nociceptive pain,** a physiologic, adaptive sensation that acts as a warning signal. There are some clinical situations, such as in acute trauma, labor, or surgery, in which it is necessary to control nociceptive pain. In these cases, the pain pathway can be interrupted either by blocking transmission with local anesthetics (see Chapter 9) or by administering high dose opioids. The opioids may be rapidly acting, such as **remifentanil** for intraoperative use, or more slowly acting, such as **morphine** for postoperative pain control.

Both peripheral inflammation and nervous system damage produce pain that is characterized by hypersensitivity to noxious and innocuous stimuli and by **spontaneous pain** (i.e., pain arising in the absence of any obvious stimulus). Understanding the mechanisms responsible for these types of clinical pain will facilitate both the appropriate use of currently available drugs and the development of novel therapeutic agents.

## CLINICAL PAIN

The ideal treatment of pain would be based on the precise mechanisms operative in a particular patient. Clinical pain syndromes may involve a combination of mechanisms, however, and there are few diagnostic tools to identify which mechanisms are responsible. Chronic pain conditions can be complicated to treat, and may demand multiple drugs to obtain the optimal therapeutic effect. Chronic inflammatory pain conditions require the use of drugs that reduce the inflammatory response; such drugs may both correct the underlying condition (disease-modifying therapy) and reduce the pain. For example, the **NSAIDs** (see Chapter 40, Pharmacology of Eicosanoids) are the first line of treatment for rheumatoid arthritis. By reducing inflammation, this intervention can prevent peripheral sensitization (see below) and reduce the release of chemical ligands that activate peripheral nerve terminals. Other disease-modifying treatments that may also reduce pain include cytokine inhibitors, such as TNFα inhibitors, and immunosuppressants.

The major agents used to treat most noninflammatory neuropathic pain conditions are generally not disease-modifying because the underlying disease processes are usually not known (e.g., fibromyalgia) or refractory to currently available treatments (e.g., neuropathic pain). Neuropathic pain associated with damage to peripheral nervous tissue, spinal cord injury, or stroke commonly requires the use of several agents to alleviate pain symptoms. Generally, in nonmalignant pain, opioids have tended to be used as a matter of last resort, because of their side effects and the potential for the development of tolerance and physical dependence (see Chapter 16, Pharmacology of Chemical Dependence and Addiction). However, in recent years, opioids have become increasingly used for the management of chronic non-cancer pain.

Severe acute pain is usually treated with opioids and fast-acting NSAIDs. For example, the pain of setting a fracture could be ameliorated effectively by the opioid remifentanil, which acts rapidly and is cleared rapidly. A more serious operative procedure, involving tissue damage that takes time to heal, may call for longer-acting agents to control postoperative pain. Acute inflammatory pain conditions, such as pancreatitis, are often treated effectively with morphine. Gout, another example of an acute inflammatory condition producing severe pain, is usually treated with indomethacin to reduce the pain immediately, while more specific disease-modifying agents are used to correct the underlying disorder over the longer term (see Chapter 46, Integrative Inflammation Pharmacology: Gout).

## PERIPHERAL SENSITIZATION

Several forms of peripheral stimuli can induce primary afferent neurons to lower their activation thresholds and increase their responsiveness (Fig. 15-5). These alterations, which constitute **peripheral sensitization,** can result in **allodynia,** in which normally innocuous stimuli are perceived as painful, and **hyperalgesia,** in which high-intensity stimuli are perceived as more painful than usual at the site of injury (zone of primary hyperalgesia). The mechanisms responsible for primary hyperalgesia involve both direct changes in transduction and indirect changes induced by the release of effector molecules. In an example of altered transduction, repeated heat activation of the TRPV1 receptor reduces its activation threshold so that it can be activated by warm, normally nonpainful, stimuli (38–40°C). The major known effectors that can produce peripheral sensitization include inflammatory mediators (bradykinin, protons, and histamine), prostaglandin $E_2$, and nerve growth factor (NGF). Prostaglandin $E_2$ acts on EP receptors, while NGF acts on TrkA receptors.

Sensitizing chemical mediators act on G protein-coupled receptors or receptor tyrosine kinases expressed on the peripheral terminals of nociceptor neurons. Activation of phospholipase C, phospholipase $A_2$, and adenylyl cyclase can occur in response to the activation of G protein-coupled receptors, such as those for bradykinin, prostaglandin E2, and adenosine. In turn, these events activate protein kinase A (PKA) or protein kinase C (PKC). Protein kinase A phosphorylates the voltage-gated sodium channel $Na_v1.8$, resulting in both a decrease in its activation threshold and an increase in the current passed when the channel opens. Protein kinase C phosphorylates TRPV1 and, thereby, increases the response of peripheral terminals to heat stimuli.

In addition to the enhancement of peripheral response caused by an outside event that produces inflammation, the peripheral terminals themselves can initiate or contribute to inflammation (neurogenic component of inflammation). Depolarization and chemical stimuli can induce the release of neuropeptides, such as substance P and CGRP, from the peripheral terminals of primary afferents. This peripheral neuropeptide release can induce the release of histamine and TNF-α from inflammatory cells. The recruitment and activation of granulocytes, accompanied by increases in local capillary diameter and permeability to plasma, results in a local inflammatory response at the site of the excited peripheral terminal.

Peripheral sensitization is an important target for clinical pain pharmacology. The nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used pain treatment. By inhibiting the activity of cyclooxygenase enzymes, NSAIDs prevent prostaglandin production and, hence, the initiation of a local inflammatory response and peripheral sensitization. There are two isoforms of cyclooxygenase, COX-1 and COX-2 (see Chapter 40). The former is constitutively active and is important in a variety of physiologic functions, such as maintenance of gastric mucosal integrity and normal

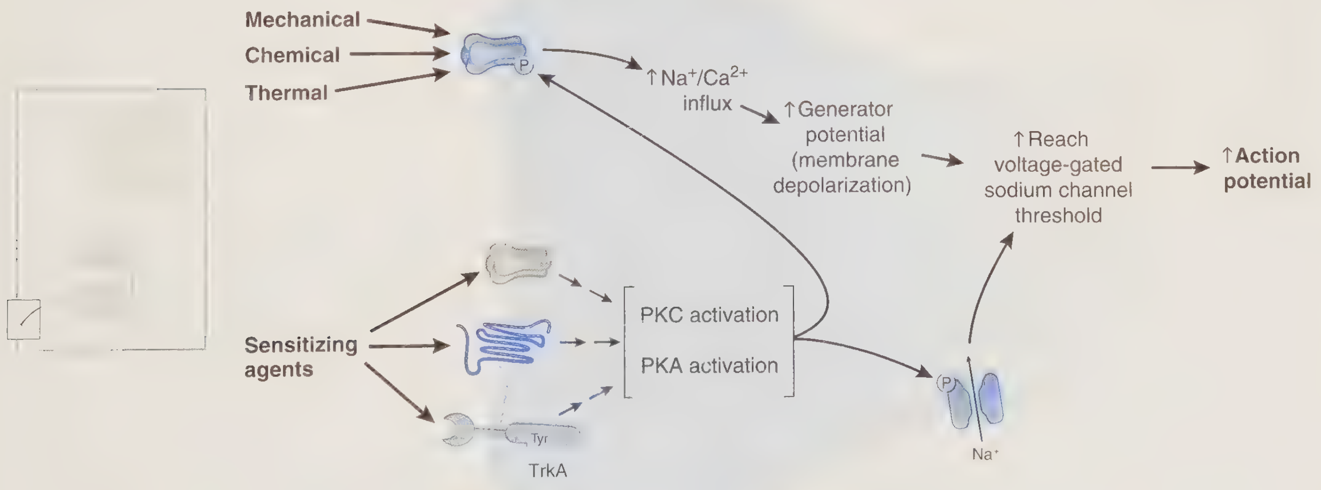


**Figure 15-5. Peripheral Sensitization.** Peripherally released sensitizing agents activate signal transduction that can increase sensitivity of the peripheral nerve terminal. Mechanisms mediating increased sensitivity include: (1) enhancement of ion influx in response to a noxious stimulus, and (2) reduction of the activation threshold of the voltage-sensitive sodium channels responsible for initiating and propagating action potentials. In the example shown, a sensitizing agent activates its G protein-coupled receptor. This receptor initiates two parallel signaling cascades. One branch activates the phospholipase C (PLC) pathway, which results in increased release of calcium from intracellular stores and in activation of protein kinase C (PKC). Both of these effects increase the ion influx in response to a nociceptive stimulus. The second branch of the signaling cascade activates adenylyl cyclase (AC), leading to increased formation of cAMP, activation of protein kinase A (PKA), and ion channel phosphorylation. Both signaling cascades serve to increase the likelihood of action potential initiation and propagation.

platelet function. COX-2 is selectively upregulated in inflammation as a response to local and systemically circulating cytokines. Selective inhibitors of COX-2, such as **rofecoxib** and **celecoxib** are, therefore, valuable in controlling inflammatory pain while decreasing some of the dangerous side effects of the nonselective NSAIDs, such as gastrointestinal bleeding. In addition to the cyclooxygenases, the transduction molecules, signaling intermediates, and sodium channels expressed at peripheral terminals may all represent targets for the development of new analgesic drugs that reduce pain hypersensitivity.

In the case of JD, peripheral sensitization was induced at the burn site. The high-intensity stimulus led to neurogenic inflammation. The associated tissue damage further potentiated inflammatory mediator release, leading to the activation of second messenger cascades that ultimately heightened peripheral terminal excitability over time.

## CENTRAL SENSITIZATION

Frequently, hyperalgesia and allodynia extend beyond the primary area of inflammation and tissue damage. Pain hypersensitivity in this region, described as the area of secondary hyperalgesia and/or allodynia, depends on alterations in sensory processing in the dorsal horn of the spinal cord. These alterations, which are a form of activity-dependent plasticity termed **central sensitization,** occur when repetitive, usually high-intensity, synaptic transmission activates intracellular signal transduction cascades in dorsal horn neurons that enhance the response to subsequent stimuli.

Several of the postsynaptic receptors expressed by dorsal horn neurons are involved in the induction of central sensitization (Fig. 15-6). These receptors include AMPA, NMDA, and metabotropic glutamate receptors, as well the neurokinin receptor NK1 for the peptide substance P, and the TrkB receptor for the neurotrophin BDNF. Upon activation of metabotropic receptors or calcium influx through NMDA channels, intracellular protein kinases, such as calcium/calmodulin kinase, PKC, and extracellular signal-related protein kinase (ERK), are activated. These effectors can, in turn, alter the function of existing membrane proteins by post-translational processing, usually by phosphorylation. For example, after NMDA receptors are phosphorylated, they open more rapidly and for longer periods in response to glutamate. Most often, central sensitization slowly subsides after the inducing stimulus ceases. However, chronic injury or inflammation can produce a state of central sensitization that persists over time.

NMDA receptor blockade can prevent both the induction and maintenance of central sensitization. For example, NMDA receptor blockade instituted preoperatively has been shown to reduce pain experienced postoperatively. A component of postoperative pain is likely attributable to NMDA receptor-dependent central sensitization associated with the intense peripheral stimulation that occurs during surgery. As another example, the NMDA blocker **ketamine** can be used in burn patients to oppose activation of sensitized NMDA receptors. NMDA receptors are widely expressed, however, and NMDA blockers such as ketamine and **dextromethorphan** can produce significant side effects, including amnesia and hallucinations. Protein kinase C or ERK are alternative targets. Although many of the signaling proteins involved in dorsal horn central sensitization are expressed in all cells, it may be possible to target treatment to the spinal cord by intrathecal or epidural injection.

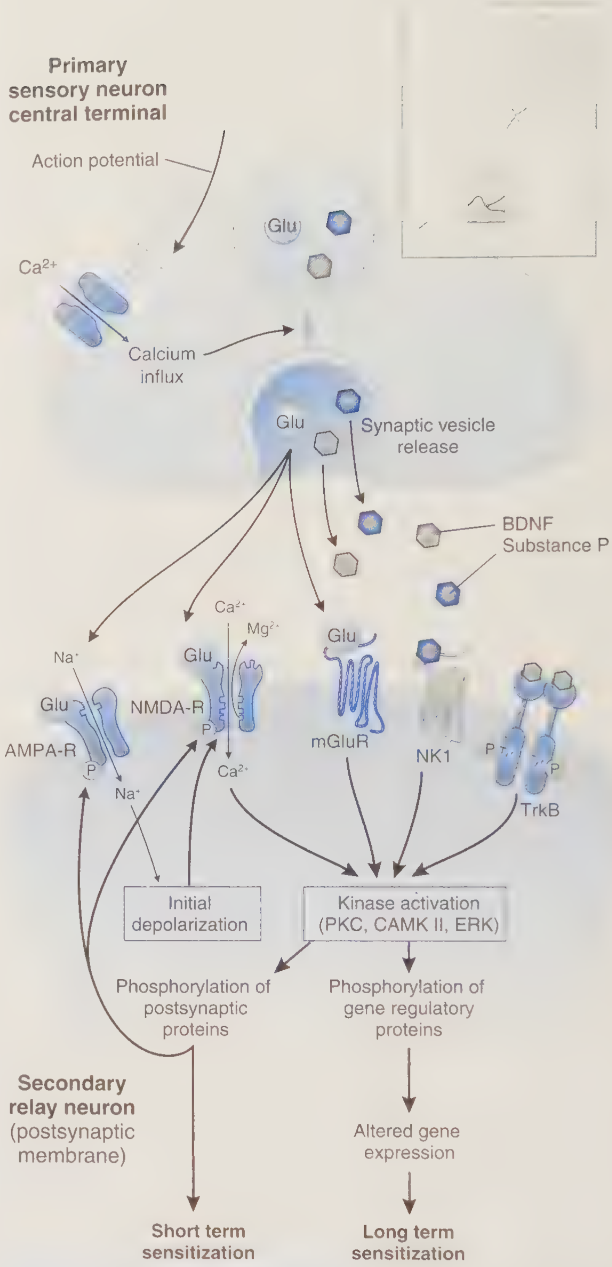


**Figure 15-6. Central Sensitization.** Sustained or intense activation of central transmission can lead to postsynaptic calcium influx, primarily through NMDA receptors. Together with a variety of neuromodulatory signals, calcium influx activates signal transduction cascades that can enhance both short-term and long-term excitability of the synapse.

The intense peripheral activation produced by JD's burn injury also led to the development of central sensitization. This effect further enhanced the lingering pain he felt at the burn site and also produced pain surrounding the burn site, outside the primary area of tissue damage and inflammation.

## NEUROPATHIC PAIN

The mechanisms responsible for the persistent pain that can occur following nerve injury involve both functional and structural alterations in the nervous system, and occur in both primary afferent neurons and the CNS (Fig. 15-7). In the periphery, changes in the physiology and transcriptional profile of primary afferent sensory neurons occur after nerve damage, contributing to neuropathic pain. These alterations are induced by combinations of positive signals, such as inflammatory cytokine release by macrophages and Schwann cells, and negative signals, such as the loss of peripheral support from neurotrophic factors. In addition, the expression pattern of sodium channels in injured primary sensory neurons changes as $Na_v$ 1.8 and $Na_v$ 1.9 are down-regulated, while $Na_v$ 1.3, which is normally not detectable in primary sensory neurons, is upregulated. $Na_v$ 1.3 channels show an accelerated recovery from inactivation, and are thought to contribute to postinjury hypersensitivity by enhancing cellular excitability. The contribution of sodium channel abnormalities to neuropathic pain is supported by the effectiveness of the sodium channel blockers, such as carbamazepine, in treating trigeminal neuralgia.

Nerve damage also promotes reorganization of synaptic connection patterns within the dorsal horn of the spinal cord. Peripheral nerve injury leads to a partial regenerative response. Because primarily C fibers are lost because of the deprivation of peripheral trophic support, the regenerating central terminals of Aβ fibers are free to invade the area normally occupied by the central terminals of C fibers. It may be possible to treat both the transcriptional changes and some of this rewiring of the circuitry with neurotrophic factors. A combination of these mechanisms would have been involved in maintaining pain in JD over the years following his operation. However, it is not currently possible to determine which set of these mechanisms are most important in an individual patient. Consequently, for each patient, it is often necessary to test more than one drug regimen before the optimal treatment for chronic pain is achieved.

## MIGRAINE

Migraine headaches, a highly prevalent spontaneous pain condition, involve certain unique pathophysiologic mechanisms that are not completely understood. The leading theory for the pathophysiology of these headaches involves three events. First, before the headache occurs, a region of neural activation followed by inactivation travels across the cortex. This phenomenon is termed **cortical spreading depression** and is correlated with the sensory disturbances of the migraine aura such as scotoma (visual field disturbances). Second, neuropeptide release, possibly evoked by cortical excitation, occurs in the dural vasculature. Third, trigeminal afferents from the dural vasculature are activated and sensitized by the local release of neuropeptides. In addition, the high degree of activity in trigeminal afferent high-threshold fibers may produce central sensitization. Thus, a migraine attack can be considered the acute manifestation of abnormal intermittent peripheral and central excitability.

## PHARMACOLOGIC CLASSES AND AGENTS

Several drug classes are widely used in the clinic for pain relief. These include **opioid receptor agonists, nonsteroidal anti-inflammatory drugs (NSAIDs)** (see Chapter 40), **tricyclic antidepressants** (see Chapter 12, Pharmacology of

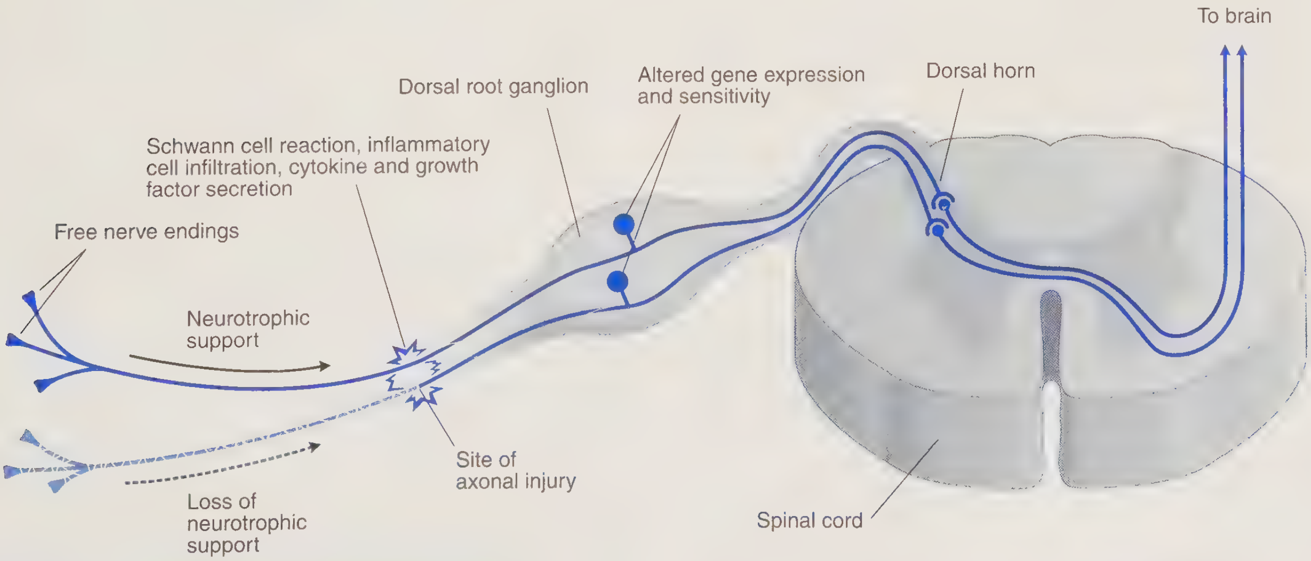

To brain
Dorsal root ganglion
Altered gene expression and sensitivity
Dorsal horn
Schwann cell reaction, inflammatory cell infiltration, cytokine and growth factor secretion
Free nerve endings
Neurotrophic support
Site of axonal injury
Loss of neurotrophic support
Spinal cord

**Figure 15-7. Schematization of Neuropathic Pain.** Nerve injury results in a combination of negative signals and positive signals that alter the physiology of the nociceptive system. The loss of neurotrophic support alters gene expression in the injured nerve fiber, whereas the release of inflammatory cytokines alters gene expression in both the injured and adjacent uninjured nerve fibers. These changes in gene expression can lead to altered sensitivity and activity of nociceptive fibers, and, thus, to the continued perception of injury that is characteristic of neuropathic pain.

Affective Disorders), **anticonvulsants** (sodium channel blockers) (see Chapter 13, Pharmacology of Abnormal Electrical Neurotransmission in the Central Nervous System), **NMDA receptor antagonists** (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission), and **adrenergic agonists.** In addition, **$5HT_1$ receptor agonists** have specific applications in the acute treatment of migraine.

## OPIOID RECEPTOR AGONISTS

Opioid receptor agonists are the primary drug class used in the management of moderate to severe pain. The naturally occurring opioid receptor agonist **morphine** has the greatest historical importance and remains in wide use, but synthetic and semisynthetic opioids add pharmacokinetic versatility. Historically, opioids have been most widely used to treat acute and cancer-related pain, but in recent years they have become one component of the management of chronic noncancer pain as well.

### Mechanism of Action and Major Side Effects

Opioid receptor agonists produce both analgesia and side effects by acting on μ-opioid receptors (Fig. 15-8). Sites of analgesic action include the brain, brainstem, spinal cord, and primary afferent peripheral terminals, as described above. Through receptors in the medullary respiratory control center, the medullary chemoreceptor zone, and the gastrointestinal tract, opioids also produce respiratory depression, vomiting, and constipation, respectively. In addition, opioids can cause sedation, confusion, dizziness, and euphoria.

Opioid use is often associated with the development of **tolerance,** in which prolonged use of a constant dose of a drug results in a decreased therapeutic effect (see Chapter 16). The molecular mechanisms responsible for tolerance remain a matter of debate, and may involve a combination of gene regulation and post-translational modification of opioid receptor activity. The development of tolerance requires either a change of analgesic or an increase in the dose or frequency of administration to maintain analgesia. **Physical dependence** can also occur, such that abrupt cessation of treatment would result in a characteristic withdrawal syndrome. Addiction, in which physical dependence is accompanied by substance abuse behavior, is a possible adverse effect of opioid administration. The incidence and prevalence of opioid addiction in patients receiving opioids for therapeutic reasons remain unknown. Balancing the risk of opioid addiction against the undertreatment of pain is a complex issue in pain management and a topic of considerable debate. In JD's case, intravenous morphine was tapered and replaced with a combined oral analgesic to prevent the onset of opioid withdrawal symptoms.

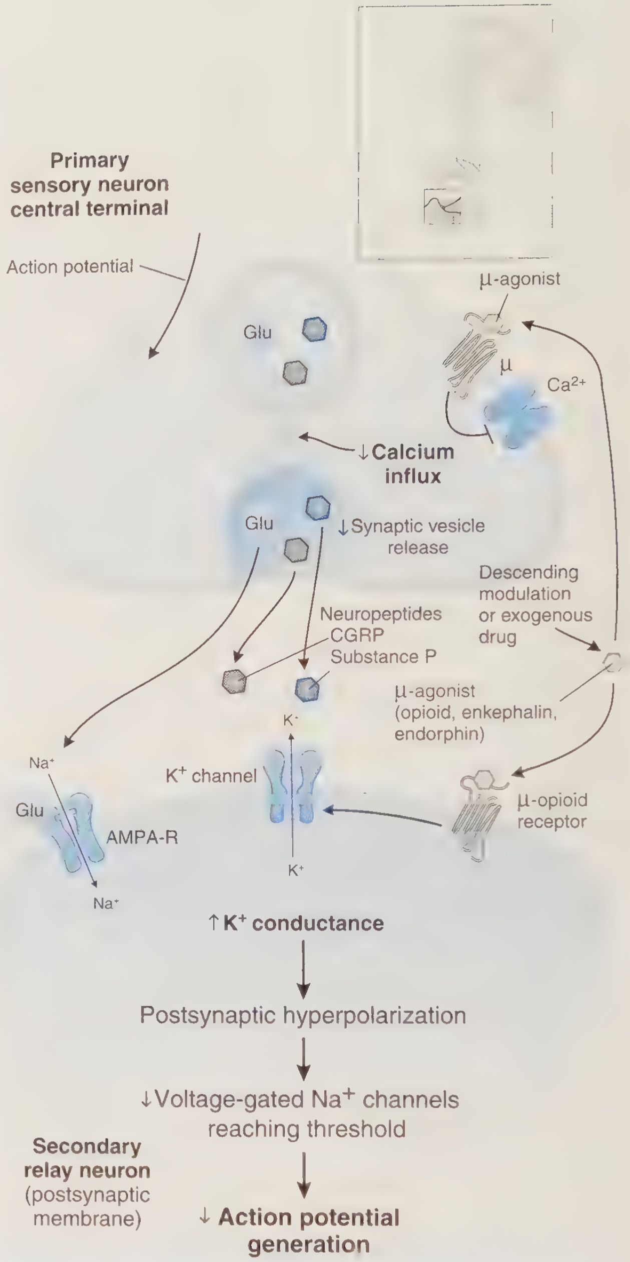

Primary sensory neuron central terminal
Action potential
Glu
μ-agonist
μ
Ca2+
↓Calcium influx
Glu
↓Synaptic vesicle release
Descending modulation or exogenous drug
Neuropeptides
CGRP
Substance P
μ-agonist (opioid, enkephalin, endorphin)
K+
Na+
K+ channel
Glu
AMPA-R
μ-opioid receptor
K+
Na+
↑K+ conductance
Postsynaptic hyperpolarization
↓Voltage-gated Na+ channels reaching threshold
Secondary relay neuron (postsynaptic membrane)
↓ Action potential generation

**Figure 15-8. Mechanism of Action of μ-Opioid Receptor Agonists in the Spinal Cord.** Activation of both presynaptic and postsynaptic μ-opioid receptors by descending and local-circuit inhibitory neurons inhibits central relaying of nociceptive stimuli. In the presynaptic terminal, μ-opioid receptor activation decreases $Ca^{2+}$ influx in response to an incoming action potential. Postsynaptic μ-opioid receptor activation increases $K^+$ conductance and, thereby, decreases the postsynaptic response to excitatory neurotransmission.

### Morphine, Codeine, and Derivatives

Morphine, **codeine (methylmorphine),** and their semisynthetic derivatives are the most widely used opioids for pain control. Morphine is typically considered the reference opioid against which others are compared. It is metabolized in the liver, and its first-pass metabolism reduces its oral availability. In the liver, morphine undergoes glucuronidation at either the 3 position (M3G) or the 6 position (M6G). While M3G is inactive, M6G has substantially greater potency than morphine itself. However, M6G is probably important only in settings that involve treatment over a sufficient period of time for it to accumulate because its increased hydrophilicity may prevent its entry into the CNS.

To meet the needs of its diverse indications, several different routes are available for administering morphine. Controlled-release oral preparations are marketed to reduce the number of daily doses required for analgesia. These formulations contain a high dose of opioid designed for release over the course of 12–24 hours. Unfortunately, because they contain high doses and are in widespread use, sustained release formulations have been associated with a high abuse potential, especially when they are illegally reformulated to deliver the entire dose at once rather than over the course of hours. Intravenous or subcutaneous morphine treatment is commonly used in patient-controlled analgesia devices, which are used in treating cancer pain and the severe acute pains of trauma, burn, surgical operation, and vaso-occlusive crisis in sickle cell disease. Finally, epidural morphine can produce highly effective analgesia by achieving high concentrations in the dorsal horn of the spinal cord. Epidural administration of this drug results in a much longer duration of action than parenteral administration, because of the time required for morphine, a relatively hydrophilic compound, to diffuse out of the CNS into the systemic circulation.

As with morphine, codeine is a naturally occurring opioid receptor agonist. Although much less effective than morphine for the treatment of pain, it is commonly used for its antitussive (that is, cough-suppressing) and antidiarrheal effects because it has considerably higher oral availability than morphine. The analgesic action of codeine results largely from its hepatic demethylation to morphine, which has substantially greater μ agonist activity. Genetic polymorphisms in the cytochrome P450 enzymes CYP2D6 and CYP3A3/4, which are responsible for demethylating codeine, may have a role in individual variations in response to treatment with codeine. The semisynthetic compounds **oxycodone** and **hydrocodone** are more effective analogues of codeine that are also orally available.

### Synthetic Agonists

Three major classes of synthetic μ receptor agonists are available, including the morphinan **levorphanol,** the **phenylpiperidines,** and the **phenylheptylamines methadone,** and **propoxyphene.** Methadone is most familiar for its use in drug addiction treatment, but it can also be used for pain management. Methadone has a 35-hour elimination half-life, which may be related to its interaction with plasma proteins. As a result of its long duration of action, methadone is often used to achieve long-lasting pain relief in patients suffering from pain in association with terminal cancer. Methadone has some NMDA receptor antagonist activity as well.

**Fentanyl,** an example of a short-acting synthetic opioid agonist, is an **anilo-phenylpiperidine** that is between 75 and 100 times more potent than morphine. Because of its high lipophilicity, fentanyl is bioavailable through several unique routes. For example, fentanyl has been formulated as a lozenge for buccal transmucosal administration, which is particularly valuable for avoiding parenteral treatment in pediatric patients. Because of its high lipid solubility, fentanyl can also be administered transdermally in the form of a patch that releases the drug slowly over time to provide long-acting systemic analgesia. **Alfentanil,** which is even more potent than fentanyl, and **sufentanil,** which is less potent, are biochemically similar to fentanyl.

**Remifentanil,** the most recently developed phenylpiperidine, exhibits distinct pharmacokinetic behavior. Remifentanil contains a methyl ester moiety that is essential for activity, but is also the substrate for the nonspecific action of numerous tissue esterases. Thus, it has unusually rapid metabolism and elimination. When administered as a continuous infusion during anesthesia, remifentanil permits unusually precise matching of the drug dose to the clinical response (see Chapter 14, General Anesthetic Pharmacology). However, the rapid offset of action demands that the use of remifentanil during anesthesia be coupled with the administration of a longer-acting drug to maintain analgesia postoperatively. In the introductory case, remifentanil was used for intraoperative analgesia during the skin graft procedure to ensure that JD did not experience pain during surgery. Morphine was added before the end of the operation to provide postoperative pain coverage. Because of remifentanil's short half-life, had morphine not been added before discontinuing the remifentanil, pain associated with surgical tissue damage would have returned immediately after surgery.

### Partial and Mixed Agonists

Although opioid receptor agonists are predominantly μ agonists, a number of drugs have also been developed as partial and mixed μ or κ agonists. These include **butorphanol** and **buprenorphine,** partial μ receptor agonists, and nalbuphine, a κ agonist with μ antagonist activity. Butorphanol and buprenorphine produce morphine-like analgesia, but with milder euphoric symptoms. Nalbuphine and similar compounds are effective analgesics because of their action at κ receptors, but are also associated with undesirable psychological dysphoria. The reduced tendency of these agents to produce euphoria may diminish the likelihood of substance abuse behavior in susceptible individuals.

### Opioid Receptor Antagonists

μ-Opioid receptor antagonists are used to reverse life-threatening side effects of opioid administration, specifically respiratory depression. **Naloxone,** one such antagonist, is a synthetic derivative of oxymorphone that is administered parenterally. Because the half-life of naloxone is shorter than that of morphine, after successful immediate treatment of an episode of respiratory depression with naloxone, it is not safe to leave the patient unattended until it is certain that the morphine no longer remains in the system. The orally administered antagonist **naltrexone** is primarily used in outpatient settings, typically for detoxification of individuals addicted to opioids (see Chapter 16).

## NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND NONOPIOID ANALGESICS

### General Features

Nonsteroidal anti-inflammatory agents inhibit the activity of **cyclooxygenase enzymes (COX-1** and **COX-2)** that are necessary for the production of prostaglandins (see Chapter 40). NSAIDs affect pain pathways in at least three different ways. First, prostaglandins reduce the activation threshold at the peripheral terminals of nociceptive primary afferent neurons (Fig. 15-9). Hence, NSAIDs, by reducing prostaglandin synthesis, reduce inflammatory hyperalgesia and allodynia directly. In addition, NSAIDs reduce the recruitment of

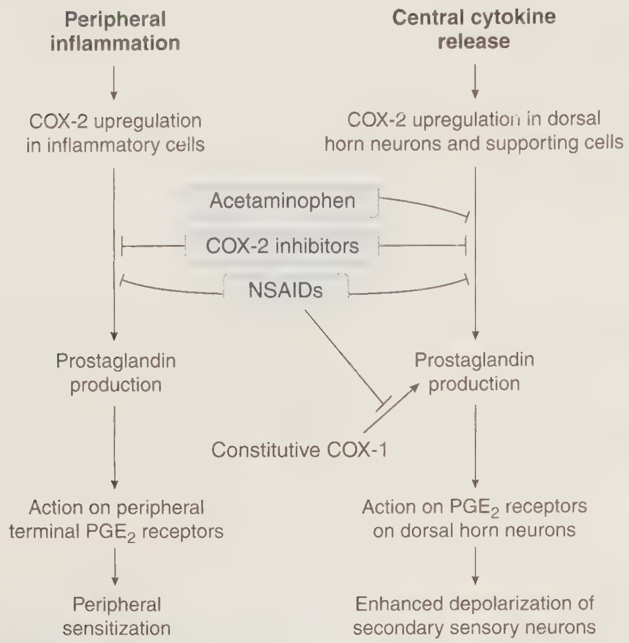

Peripheral inflammation
Central cytokine release
COX-2 upregulation in inflammatory cells
COX-2 upregulation in dorsal horn neurons and supporting cells
Acetaminophen
COX-2 inhibitors
NSAIDs
Prostaglandin production
Prostaglandin production
Constitutive COX-1
Action on peripheral terminal PGE2 receptors
Action on PGE2 receptors on dorsal horn neurons
Peripheral sensitization
Enhanced depolarization of secondary sensory neurons

**Figure 15-9. Mechanism of Analgesic Action of Cyclooxygenase Inhibitors.** Inflammatory states are often associated with the production of prostaglandins, which are important mediators of both peripheral (left) and central (right) pain sensitization. In the periphery, prostaglandins produced by inflammatory cells sensitize peripheral nerve terminal prostaglandin (EP) receptors, making them more responsive to a painful stimulus. In central pain pathways, cytokines released in response to inflammation induce prostaglandin production in the dorsal horn of the spinal cord. These prostaglandins sensitize secondary nociceptive neurons and, thereby, increase the perception of pain. Cyclooxygenase (COX) inhibitors, including nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors, both block peripheral and central sensitization mediated by the prostanoids that are released in inflammation and reduce the extent of inflammation.

leukocytes, which produce other inflammatory mediators. Finally, prostaglandins act as pain-producing neuromodulators in the spinal cord dorsal horn, where their production may be prevented by NSAIDs. Because **acetaminophen** and NSAIDs act through mechanisms different from the opioids, NSAID–opioid or acetaminophen–opioid combinations can act synergistically to reduce pain. NSAIDs and COX-2 inhibitors act both peripherally and centrally, whereas acetaminophen acts only centrally.

Like the opioids, nonselective COX-inhibiting NSAIDs have deleterious side effects, particularly injury to the gastric mucosa and the kidneys. In some settings, these side effects can be minimized by cotreatment with such other drugs as **misoprostol,** which helps replace the prostaglandin activity essential for the normal function of the gastric mucosa, although this drug carries its own set of side effects (diarrhea, uterine contraction). It is thought that the anti-inflammatory and analgesic effects of the NSAIDs are primarily attributable to inhibition of COX-2, an inducible enzyme active in inflammatory states, while the side effects are primarily attributable to inhibition of COX-1, a constitutive enzyme responsible for the production of prostanoids involved in physiologic tissue maintenance and vascular regulation. However, this view may be an oversimplification because COX-2 may be induced to support COX-1 activity in the setting of gastric mucosal injury, while COX-1 may produce prostaglandins in tandem with COX-2 in inflammatory states.

## Specific Agents

There are several major classes of NSAIDs, including the **salicylates (aspirin** or **acetylsalicylate), para-aminophenols (acetaminophen), indole acetic acid** derivatives **(indomethacin), pyrrole acetic acid** derivatives **(diclofenac), propionic acid** derivatives **(ibuprofen),** and **benzothiazines (piroxicam).** Finally, the recently developed **COX-2 selective agents celecoxib, rofecoxib,** and **valdecoxib** may prove valuable for producing analgesia while decreasing some of the adverse effects associated with chronic NSAID use. Representative agents are discussed here; further information on their specific anti-inflammatory uses may be found in the Chapter 40, Pharmacology of Eicosanoids.

- Acetylsalicylic acid (aspirin) acts by covalently acetylating the cyclooxygenase active site in both COX-1 and COX-2. Aspirin is rapidly absorbed and distributed throughout the body. Chronic aspirin use can produce gastric irritation and erosion, hemorrhage, vomiting, and renal tubular necrosis. Aspirin is of great value in the treatment of mild or moderate pain.
- Acetaminophen (paracetamol) preferentially reduces central prostaglandin synthesis by an unknown mechanism, and as a result produces analgesia and antipyresis but has relatively little anti-inflammatory efficacy. Acetaminophen is frequently combined with weak opioids for the treatment of moderate pain, and preparations featuring acetaminophen combined with codeine, hydrocodone, oxycodone, pentazocine, or propoxyphene are available.
- The coxibs are a class of recently introduced COX-2 selective agents. Currently, celecoxib, rofecoxib, and valdecoxib are in clinical use. These drugs were originally reserved for patients who required NSAIDs but were at high risk for developing gastrointestinal, renal, or hematologic side effects. Celecoxib and rofecoxib are associated with a reduced incidence of gastric ulcers. In a phase 3 clinical trial, patients treated with rofecoxib had a higher rate of myocardial infarction than patients treated with the nonspecific cyclooxygenase inhibitor **naproxen.** It has not yet been determined whether the result was attributable to a specific harmful effect of rofecoxib, a general deleterious effect of COX-2 inhibition, or a cardioprotective effect of naxopren.
- The widely used compound ibuprofen is a derivative of propionic acid. Used primarily for analgesia and anti-inflammatory action, it also is an antipyretic, and has a relatively lower incidence of side effects than aspirin.
- The pyrrole acetic acid derivatives diclofenac and ketorolac are used to treat moderate to severe pain states. Ketorolac can be administered orally or parenterally, while diclofenac is available in oral formulations. Both agents carry a risk of severe side effects such as anaphylaxis, acute renal failure, Stevens–Johnson syn-

drome (a diffuse life-threatening rash involving the skin and mucous membranes), and gastrointestinal bleeding.

## ANTIDEPRESSANTS

Drugs originally developed for treating depression are widely used as adjuvant therapy in pain management, particularly for treatment of chronic pain conditions. It is thought that antidepressants produce analgesia both by increasing the activity of noradrenergic and serotonergic projections descending from the brain to the spinal cord (which are normally involved in producing antinociceptive states) and by blocking sodium channels. Many specific agents have been studied in clinical trials for their efficacy in specific pain conditions. A finding that antidepressants are efficacious in any one clinical pain condition, however, does not generalize well to other pain conditions. For example, diabetic neuropathy and postherpetic neuralgia respond relatively well to therapy with an antidepressant, whereas antidepressants have a minimal effect on chronic low back pain. In general, the least selective agents (i.e., those with the broadest neurochemical effects), such as the tricyclics **amitriptyline, nortriptyline,** and **imipramine,** have been more effective than the selective noradrenergic reuptake blockers **desipramine** and **maprotiline,** while serotonin-selective reuptake inhibitors such as **paroxetine, fluoxetine,** and **citalopram** are the least effective. The use of these drugs in mood disorders is discussed in Chapter 12.

## ANTICONVULSANTS AND ANTIARRHYTHMICS

A number of pharmacologic agents used to control the excessive cellular excitability that leads to seizures (see Chapter 13) or cardiac arrhythmias (see Chapter 17) can also be used to manage the symptoms of some chronic pain conditions. In the search for drugs that can produce analgesia, a number of these agents have been tested on the basis of their ability to reduce neuronal excitability. Of these, the most clinically valuable are currently the anticonvulsants **gabapentin, lamotrigine,** and **carbamazepine.**

Gabapentin has recently become widely used for the management of chronic pain. It was originally developed as a structural analogue of GABA, but it does not bind to the GABA receptor and does not affect the metabolism or reuptake of GABA. Gabapentin can bind to the $\alpha 2\delta$ subunit of voltage-dependent calcium channels, but it remains to be determined whether this is the site responsible for the effects of gabapentin on neuronal activity and on pain in patients. Randomized clinical trials in diabetic neuropathy and trigeminal neuralgia show that gabapentin is superior to placebo in reducing subjectively reported pain. Gabapentin is associated with several adverse effects, particularly dizziness, somnolence, confusion, and ataxia. It is widely thought that gabapentin's side effects are milder than those of amitriptyline, but this has not yet been proved in a clinical trial. In the introductory case, gabapentin reduced JD's spontaneous paroxysmal pain, probably by reducing aberrant neuronal excitability. However, the exact molecular mechanism of gabapentin's action remains in question.

Carbamazepine acts to block sodium channels, and is used primarily to treat trigeminal neuralgia. Lamotrigine, an antiepileptic drug that is also a sodium channel blocker, reduces the painful sensory symptoms that can occur in neuropathy, stroke, multiple sclerosis, and phantom limb. The use of mexiletine, an antiarrhythmic, is limited by gastrointestinal side effects. Lidocaine, a use-dependent sodium channel blocker, is typically used as a local anesthetic (see Chapter 9). It can also be used for regional pain management when administered intravenously.

## NMDA RECEPTOR ANTAGONISTS

Because of the critical role of NMDA receptors in the induction and maintenance of central sensitization, antagonists of the NMDA receptor are currently under investigation for use in pain treatment. Two currently available drugs act as antagonists at the NMDA receptor, and both of these drugs, the anesthetic **ketamine** and the antitussive **dextromethorphan,** effectively reduce chronic pain symptoms and postoperative pain. Ketamine use is severely limited by its psychomimetic effects. Dextromethorphan, when used at the relatively high doses required for clinically observable analgesia, also produces dizziness, fatigue, confusion, and psychomimetic effects.

## ADRENERGIC AGONISTS

Stimulation of $\alpha_2$-adrenergic receptors in the dorsal horn of the spinal cord produces an antinociceptive state. $\alpha_2$-Adrenergic agonists, therefore, may have therapeutic utility as analgesics. The $\alpha_2$-agonist **clonidine** has been used systemically, epidurally, intrathecally, and topically, and seems to produce analgesia in both acute and chronic pain states. Clonidine does, however, cause postural hypotension; this side effect limits its usefulness in pain control.

## MIGRAINE THERAPY

The treatment of pain associated with migraine has features distinct from the treatment of other pain conditions. In many, but not all, patients, an effective treatment for migraine is the **triptan** class of serotonin receptor agonists, whose most well-studied example is **sumatriptan.** The triptans are selective for the 5-$HT_{1B}$ and 5-$HT_{1D}$ subtypes. These drugs reduce both sensory activation in the periphery and nociceptive transmission in the brainstem trigeminal nucleus, where they diminish central sensitization. The triptans also cause vasoconstriction, opposing the vasodilation thought to be involved in the pathophysiology of migraine attacks. It remains unclear whether the vasoconstriction is helpful in producing the antimigraine actions of these drugs, however. Furthermore, as a result of this vasoconstrictive effect, the triptans can be dangerous in patients with coronary heart disease. The triptans can reduce the pain and other symptoms associated with acute migraine attack, and have replaced the vasoconstrictive agent **ergotamine tartrate** in the treatment of migraine. Sumatriptan can be administered subcutaneously, orally, or by nasal inhalation. Several other orally administered agents in the triptan class have recently become available, including **zolmitriptan, naratriptan,** and **rizatriptan** (see Drug Summary Table). NSAIDs and opioids also have activity and some utility for treatment of acute migraine headaches. During an attack, migraine patients often experience gastric stasis that can reduce the bioavailability of oral medications.

Although the triptans are relatively effective in ameliorating the acute symptoms of migraine, different drugs are used to reduce the frequency of attacks. Numerous drugs are used for migraine prophylaxis, including β-adrenergic blockers, valproic acid, serotonin antagonists, and calcium channel blockers. These agents are generally chosen based on the severity and frequency of the migraine attacks, the cost of the drug, and the side effects of the drug in the context of the individual patient. None has been shown to have a high level of efficacy, and new drugs must be developed for more effective migraine prophylaxis.

## *Conclusion and Future Directions*

Because of the limited efficacy of any single drug, it is common in clinical practice to use a polypharmacy approach to managing pain. In combination, several drugs that are only moderately effective as single agents can have additive or supra-additive effects. This is largely a consequence of the multiple processing events and mechanisms responsible for producing pain: intervention at several steps may be required to achieve adequate analgesia (Fig. 15-10). Because many drugs used to treat pain are also active systemically and/or in parts of the nervous system that are not related to somatic sensation, analgesics can produce deleterious side effects. One approach to limiting toxicity is to use localized (nonsystemic) forms of drug delivery. In particular, epidural and topical delivery limit the drug administered to a local site of action. Many of the opioids are short-acting and must be administered frequently to patients in severe pain. Modes of drug delivery have also been developed to optimize the pharmacokinetics of the short-acting opioids; these methods include transdermal and buccal dosage forms, patient-controlled analgesia devices, and controlled-release oral preparations. Patient-controlled devices ensure that patients do not suffer pain because of waning drug effects; whereas instrumental controls can effectively prevent overdose. At the present time, however, these technologies are suitable only for inpatient treatment.

Most of the currently available analgesics have been derived from empiric observation (opioids, NSAIDs, and local anesthetics) or serendipity (anticonvulsants). Now that the mechanisms responsible for pain are being explored at a molecular level, many new targets are being revealed that are likely to lead to quite different classes of analgesics. It is hoped that drugs active at these new targets will achieve greater efficacy and have fewer side effects than current therapies. Effective pain management approaches must rely not only on pharmacologic intervention; psychotherapy, physical therapy and rehabilitation, and, in some very limited situations, surgical approaches may also have a role. The placebo reaction and a variety of mind–body therapies can also produce analgesia and may explain the limited success produced by such treatments as acupuncture and homeopathy. The mechanisms of these responses are unknown, however, and the effects are often unpredictable, modest, and of short duration. The growing complexity of pain management has spawned specialized pain services for inpatient pain control, as well as pain clinics and centers for the outpatient management of chronic pain.

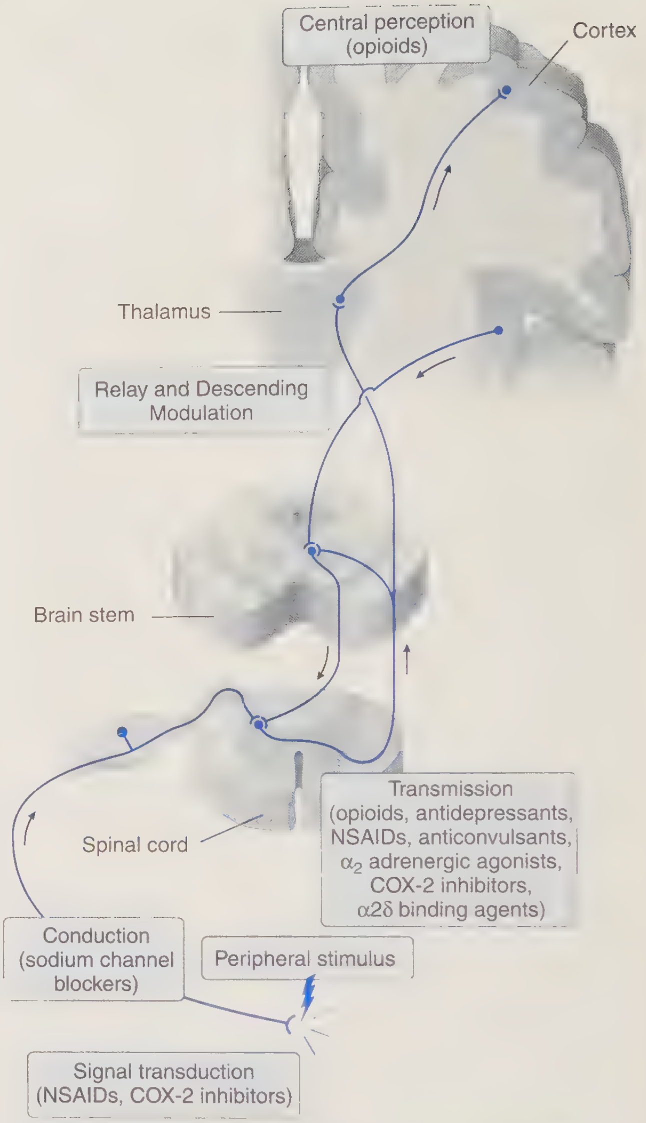

Central perception (opioids)
Cortex
Thalamus
Relay and Descending Modulation
Brain stem
Transmission (opioids, antidepressants, NSAIDs, anticonvulsants, $\alpha_2$ adrenergic agonists, COX-2 inhibitors, $\alpha 2\delta$ binding agents)
Spinal cord
Conduction (sodium channel blockers)
Peripheral stimulus
Signal transduction (NSAIDs, COX-2 inhibitors)

**Figure 15-10. Summary of the Sites of Action of the Major Drug Classes Used for Pain Management.** Analgesics target various steps in pain perception, from the initiation of a pain stimulus to the central perception of that pain. Nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors modulate the initial membrane depolarization (signal transduction) in response to a peripheral stimulus. Sodium channel blockers decrease action potential conduction in nociceptive fibers. Opioids, antidepressants, NSAIDs, anticonvulsants, and $\alpha_2$-adrenergic agonists all modulate transmission of pain sensation in the spinal cord by decreasing the signal relayed from peripheral to central pain pathways. Opioids also modulate the central perception of painful stimuli. The multiple sites of action of analgesics allow a combination drug approach to be used in pain management. For example, moderate pain is often treated with combinations of opioids and NSAIDs. Because these drugs have different mechanisms and sites of action, the combination of the drugs is more effective than either drug alone.

## *Acknowledgments*

The authors thank Salahadin Abdi, MD; Rami Burstein, PhD; Carl Rosow, MD, PhD; and Joachim Scholz, M.D. for their valuable comments.

## *Suggested Readings*

Fitzgerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001;345:433–442. (*Discusses the effectiveness and merits of COX-2 inhibitors.*)

Julius D, Basbaum AI. 2001. Molecular mechanisms of nociception. Nature 2001;413:203–210. (*Discussion of pain mechanisms, focusing on the peripheral terminal.*)

Loeser JD, ed. 2001. Bonica's Management of Pain. Philadelphia: Lippincott Williams & Wilkins, 2001. (*Comprehensive text on pain mechanisms, disorders associated with pain, and treatment of pain.*)

Mao J, Chen LL. Gabapentin in pain management. Anesth Analg 2000;91:680–687. (*Review synthesizing basic and clinical evidence about the use of gabapentin in the treatment of pain.*)

Mendell JR, Sahenk Z. Painful sensory neuropathy. N Engl J Med 2003;348:1243–1255. (*Clinically oriented discussion of the diagnosis, pathophysiology, and treatment of painful sensory neuropathy.*)

Portenoy RK. 2000. Current pharmacotherapy of chronic pain. J Pain Symptom Manage 2000;19:S15–S20. (*Reviews some of the major modalities of pain treatment.*)

Rosow CE. An overview of remifentanil. Anesth Analg 1999; 89: S1–S3. (*Detailed discussion of remifentanil, a recently developed opioid agonist.*)

Scholz J, Woolf CJ. Can we conquer pain? Nature Neurosci 2002; 5Suppl:1062–1067.

Waxman SG. Transcriptional channelopathies: An emerging class of disorders. Nature Reviews: Neurosci 2001;2:652–659. (*Reviews pathologic states associated with transcriptional changes in ion channel gene expression; pain is included as an example.*)

Woolf CJ, Salter MW. Neuronal plasticity: Increasing the gain in pain. Science 2000;288: 1765–1769. (*Discusses regulated sensitivity of the nociceptive system.*)

Yaksh TL. Central pharmacology of nociceptive transmission. In: Wall PD, Melzack R, eds. Textbook of Pain. New York: Churchill Livingstone, 1999. (*Contains a discussion of dorsal horn neurotransmission as it relates to pain.*)

**Drug Summary Table** **Pharmacology of Analgesia**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **μ-OPIOID RECEPTOR AGONISTS**<br>*Mechanism—Inhibit neurotransmission by activating the μ-opioid receptor* | | | | |
| **Morphine**<br>**Oxycodone**<br>**Hydrocodone** | Severe pain | All μ-agonists can cause:<br>Respiratory depression<br>Constipation<br>Nausea and vomiting<br>Sedation<br>Euphoria<br>Peripheral vasodilation<br>Urinary retention<br>Spasm-sphincter of Oddi<br>Pruritus<br>Physical dependence<br>Tolerance<br>Miosis | All μ-agonists are relatively contraindicated in:<br>Impaired pulmonary function<br>Asthma<br>Hepatic or renal disease<br>Hypotension<br>All μ-agonists can interact with:<br>Phenothiazines (enhanced respiratory depression, sedation)<br>Antidepressants (enhanced analgesia)<br>MAO inhibitors (contraindicated with meperidine) | |
| **Levorphanol** | Severe pain | | | |
| **Meperidine** | Severe pain | | | |
| **Methadone** | Severe pain<br>Opioid addiction | | | Hepatic enzyme inducers accelerate metabolism of methadone |
| **Codeine** | Mild pain | | | |
| **Propoxyphene** | Mild pain | | | Propoxyphene is less potent than codeine |
| **Fentanyl**<br>**Alfentanil**<br>**Sufentanil**<br>**Remifentanil** | Severe pain | | | Fentanyl, afentanil, sufentanil, and remifentanil do not cause vasodilation<br>Sufentanil has rapid onset, slower offset<br>Remifentanil has rapid onset, rapid offset |
| **μ-OPIOID RECEPTOR PARTIAL AGONISTS**<br>*Mechanism—Activate the μ-opioid receptor* | | | | |
| **Butorphanol**<br>**Buprenorphine** | Severe pain | μ-Agonist side effects (see above) | | Less efficacious than μ-opioid receptor full agonists (i.e., effects plateau with increasing dose) |
| **μ-OPIOID RECEPTOR ANTAGONISTS**<br>*Mechanism—Bind to the μ-opioid receptor without eliciting receptor activation; block the effects of exogenous or endogenous opioids* | | | | |
| **Naloxone** | Reversal of acute opioid toxicity | Precipitates acute opioid withdrawal | | Parenteral use only |
| **Naltrexone** | Opioid addiction | | | Oral use, slower onset than naloxone |
| **NONOPIOID ANALGESICS**<br>*Mechanism—Block prostaglandin production* | | | | |
| **Acetaminophen** | Mild pain | Hepatotoxicity | Alcohol can ↑ hepatotoxicity | Not an anti-inflammatory drug |
| **Acetylsalicylic acid (aspirin)**<br>**Naproxen**<br>**Ibuprofen** | Mild inflammatory pain<br>Migraine | Renal failure<br>Gastric irritation/ulceration | Hemorrhagic disorders<br>Known aspirin sensitivity | Aspirin overdose can cause CNS toxicity, nausea, and respiratory stimulation |
| **Indomethacin**<br>**Diclofenac**<br>**Piroxicam** | Moderate inflammatory pain<br>Low back pain | Renal failure<br>Gastric irritation/ulceration | Asthma<br>Pregnancy (can delay labor) | |
| **Celecoxib**<br>**Rofecoxib**<br>**Valdecoxib** | Moderate inflammatory pain, particularly when chronic | Altered renal function<br>Possible prothrombotic effect | Pregnancy (can delay labor) | COX-2-selective inhibitors |

| | | | | |
|---|---|---|---|---|
| **TRICYCLIC ANTIDEPRESSANTS (TCAs)**<br>*Mechanism—Promote serotonergic and noradrenergic neurotransmission by inhibiting neurotransmitter reuptake* | | | | |
| **Amitriptyline**<br>**Nortriptyline**<br>**Imipramine**<br>**Desipramine** | All TCAs can be used in neuropathic pain | All TCAs can cause:<br>Cardiac arrhythmias<br>Orthostatic hypotension<br>Antimuscarinic effects<br>Sedation, weight gain<br>Insomnia<br>Sexual disturbances | All TCAs are contraindicated in cardiac arrhythmia and cardiac conduction defect<br>Alcohol can exacerbate sedation | |
| **ANTICONVULSANTS AND ANTIARRHYTHMICS**<br>*Mechanism—Block action potential production or conduction* | | | | |
| **Carbamazepine** | Neuropathic pain, especially trigeminal neuralgia | Sedation, arrhythmia, dizziness, skin rash | Induces hepatic microsomal enzymes<br>Accelerates warfarin metabolism<br>Can induce seizure if dose ↓ | In rare cases, can cause agranulocytosis |
| **Gabapentin** | Neuropathic pain | Dizziness, ataxia, sedation, nausea | Can induce seizure if dose ↓ | |
| **Lamotrigine** | Neuropathic pain | Skin rash, sedation, double vision, dizziness, nausea | Can induce seizure if dose ↓ | Risk of skin rash is reduced by slowly increasing the dose |
| **Mexiletine** | Neuropathic pain, especially diabetic neuropathy | Tremor, lethargy, blurred vision, nausea | Cardiac arrhythmias | |
| **NMDA RECEPTOR ANTAGONISTS**<br>*Mechanism—Block NMDA receptor-dependent postsynaptic depolarization* | | | | |
| **Ketamine** | Neuropathic pain | Cardiovascular stimulation<br>↑ intracranial pressure<br>Psychosis | | |
| **Dextromethorphan** | Neuropathic pain | Dizziness, confusion, fatigue | | |
| **5-HT$_{1D}$ SEROTONIN RECEPTOR AGONISTS**<br>*Mechanism—Induce cerebrovascular vasoconstriction* | | | | |
| **Sumatriptan**<br>**Rizatriptan**<br>**Naratriptan**<br>**Zolmitriptan**<br>**Almotriptan**<br>**Eletriptan** | All 5HT$_{1D}$-agonists can be used in acute migraine attacks | All 5HT$_{1D}$-agonists can cause:<br>Paresthesias<br>Sensations of warmth<br>Neck stiffness<br>Coronary vasospasm | All 5HT$_{1D}$-agonists are contraindicated in patients with vascular disease, due to induction of vasospasm | |

# 16

# Pharmacology of Chemical Dependence and Addiction

Joshua M. Galanter and Alan A. Wartenberg

Introduction
Case
Definitions
Mechanisms of Tolerance, Dependence, and Addiction
  Mechanisms of Tolerance
  Mechanisms of Physical Dependence
  Mechanisms of Psychologic Dependence
  Mechanisms of Addiction
    *Variables Affecting the Development of Addiction*
Drugs of Abuse
  Commonly Prescribed Therapeutics
    *Opioids*
    *Benzodiazepines and Barbiturates*
  Drugs Related to Therapeutics
    *Heroin*
    *Alcohol*
    *Nicotine*
    *Cocaine and Amphetamine*
  Drugs of Abuse Affecting Other Receptors
Treatments for Addiction and Dependence
  Detoxification
  Social Treatments
  Pharmacologic Treatment
Conclusion and Future Directions
Acknowledgment
Suggested Readings

## INTRODUCTION

The previous chapters in this section have focused on pharmacologic agents used for therapeutic purposes in the central nervous system (CNS). This chapter considers pharmacologic agents that cause chemical dependence and addiction. It is important to note that chemical dependence can follow administration of any therapeutic, including agents that affect processes outside the CNS. For example, rebound sodium retention often follows the discontinuation of loop diuretics and is caused by the body's attempt to re-establish homeostasis. Addiction, on the other hand, involves changes in neuronal pathways that cause the individual's priorities to be centered on harmful drug-seeking and drug-using behaviors at the expense of normal social interactions.

This chapter first defines tolerance, dependence, and withdrawal, and explores the mechanisms by which they arise. Drug abuse, misuse, and addiction are then discussed, with an emphasis on the possible mechanisms that underlie craving and relapse. Although the chapter does not describe exhaustively the mechanisms of action of all drugs of abuse, selected representative drugs are considered mechanistically, and the mechanisms of the important remaining drugs are summarized in Table 16-1. Finally, the therapeutic strategies—both pharmacologic and social—used to combat drug dependence and addiction are discussed. Some strategies are common to all drugs of abuse and misuse. Because these efforts are not always successful (as in the case below), research into the causes and treatments of drug dependence and addiction must continue.

## ■ Case

J.B., a 25-year-old man with a history of heavy heroin use, is brought to an emergency department in suburban Phoenix with an 8-hour history of increasing nausea, vomiting, diarrhea, muscle aches, and anxiety. Mr. B explains that he is trying to "kick the habit" and that his last "hit" was approximately 24 hours ago. He expresses an intense craving for heroin and is extremely fidgety and uncomfortable. On physical examination, he has a temperature of 103°F, enlarged pupils, a blood pressure of 160/95, and a heart rate of 108. He is irritable and exquisitely sensitive to touch, and his responses to painful stimuli, such as a pinprick, are out of proportion to the intensity of the stimulus. Mr. B is given 20 mg of methadone, a long-acting opioid. He becomes slightly more comfortable and is given a second dose of 20 mg, after which he is noticeably more

**TABLE 16-1** Major Drugs of Abuse

| DRUG CLASS | EXAMPLES | RECEPTOR (ACTION) | CLINICAL SIGNS | NOTES |
|---|---|---|---|---|
| Opioids | Morphine<br>Heroin<br>Codeine<br>Oxycodone | μ-Opioid (agonist) | Euphoria, followed by sedation, respiratory depression | Used therapeutically as analgesics (except for heroin) |
| Benzodiazepines | Triazolam<br>Lorazapam<br>Diazepam | $\text{GABA}_A$ (modulator) | Sedation, respiratory depression | Used therapeutically as anxiolytics, sedatives |
| Barbiturates | Phenobarbital<br>Pentobarbital | $\text{GABA}_A$ (modulator) | Sedation, respiratory depression | Used therapeutically as anxiolytics and sedatives; greater danger of overdose than benzodiazepines |
| Alcohol | Ethanol | $\text{GABA}_A$ (modulator), NMDA antagonist | Intoxication, sedation, memory loss | Legal in many countries |
| Nicotine | Tobacco | Nicotinic ACh (agonist) | Alertness, muscle relaxation | Legal in many countries |
| Psychostimulants | Cocaine<br>Amphetamine | Dopamine, adrenergic, serotonin (reuptake inhibitor) | Euphoria, alertness, hypertension, paranoia | Amphetamines also reverse the reuptake transporter |
| Caffeine | Coffee | Adenosine (antagonist) | Alertness, tremulousness | Generally legal, addiction rare |
| Cannabinoids | Cannabis | CB1, CB2 (agonist) | Changes in mood, hunger, giddiness | |
| Phencyclidine (PCP) | N/A | NMDA (antagonist) | Hallucinations, hostile behavior | |
| Phenylethylamines | MDMA (Ecstasy), MDA | Serotonin, dopamine, adrenergic (reuptake inhibitors, multiple actions) | Euphoria, alertness, hypertension, hallucinations | Structurally related to amphetamines, effects similar to psychedelic agents |
| Psychedelic agents | LSD<br>DMT<br>Psilocybin | $5\text{-HT}_2$ (partial agonist) | Hallucinations | |
| Inhalants | Toluene<br>Amyl nitrate<br>Nitrous oxide | Unknown | Dizziness, intoxication | |

comfortable and the worst of his symptoms abate. Mr. B is then admitted to an inpatient detoxification center to complete a 28-day "detox" program. Over the course of the next week, his methadone dose is decreased by approximately 20% per day. Mr. B is enrolled in a Narcotics Anonymous (NA) program, where he tells the tale of his addiction. It had started out slowly, with only a few hits of heroin per month, "on special occasions," as he puts it. Over time, however, he had found that the high he got from the drug wasn't as intense as it had been when he first started, and he had found himself shooting (injecting intravenously) both larger amounts and more frequently. Eventually, he was shooting twice a day and felt as if he was "trapped" by the drug.

Although Mr. B finds the sessions at NA useful, his attendance is sporadic. Over the next few weeks he experiences cyclical changes in weight, alternating periods of insomnia and anxiety, and craving for heroin despite opioid-free urine tests. Two months later he relapses, and is again shooting heroin twice daily.

## QUESTIONS

1. What caused Mr. B's physical symptoms and signs (i.e., nausea, vomiting, fever, enlarged pupils, hypertension, etc.) on his initial visit to the emergency department?
2. Why was Mr. B treated for heroin withdrawal with another opioid (methadone)?
3. Why did Mr. B find that, over time, the effect of heroin was less intense than when he first started shooting?
4. Why did Mr. B experience intense cravings for heroin after his physiologic symptoms abated?
5. How can programs such as Narcotics Anonymous help treat addiction?

## DEFINITIONS

Before describing the various drugs of abuse and the possible mechanisms of addiction and dependence, it is helpful to discuss the definitions of several important terms. Broadly speaking, the terms used in this chapter can be separated into two categories: those based on social constructs and those based on physiologic constructs.

The terms abuse and misuse have social implications and, possibly for this reason, are often used inappropriately. The American Psychiatric Association (APA) defines the term **drug abuse** as "a maladaptive pattern of substance use leading to clinically significant impairment or distress" associated with any of a number of symptoms. The term is commonly used in the case of *nonprescribed* substances, although the APA does not make this distinction. **Drug misuse,** not formally defined by the APA, commonly refers to the improper use of *prescribed* compounds or the use of such compounds for nontherapeutic purposes. Thus, ethanol and nicotine, which are not commonly prescribed, are considered drugs of abuse. On the other hand, the use of morphine or a benzodiazepine for purposes other than its therapeutic indication, or at a higher or more frequent dose than indicated, would represent a misuse of that drug.

These terms are largely social constructs, and many cases blur the line between abuse and misuse. For example, marijuana, long considered a drug of abuse, is now finding legitimate therapeutic uses as an antiemetic for patients undergoing cancer chemotherapy; several states permit health care professionals to prescribe marijuana for that purpose. Moreover, physicians occasionally prescribe ethanol or nicotine; ethanol is a competitive inhibitor of alcohol dehydrogenase that can be used as a treatment for ethylene glycol (antifreeze) or methanol poisoning, while nicotine (in the form of a patch or a gum) can be used to combat the effects of nicotine withdrawal. In contrast, some drugs that were once used therapeutically have been displaced by safer or more effective alternatives. More importantly, the distinction between a "therapeutic" and a "nontherapeutic" use of a drug is imprecise, because evidence suggests that much drug abuse is rooted in an attempt to self-medicate. Thus, ethanol may be self-administered as an anxiolytic, even though such use is not monitored or endorsed by a physician, whereas caffeine, perhaps the most widely used substance that is active in the CNS, is self-administered as a stimulant to combat drowsiness. Given these ambiguities, it may be safer to follow the APA definition of drug abuse and not to make rigid distinctions between "therapeutic" and "nontherapeutic" uses of a drug.

Because of their physiologic bases, the terms tolerance, dependence, and withdrawal are easier to define. In addition, these terms apply equally well to physician-prescribed and nonprescribed drugs. **Tolerance** refers to the decreased effect of a drug that develops with continued use. The first administration of a drug produces a characteristic dose–response curve; after repeated administrations of the same drug, however, the dose–response curve shifts to the right as *larger doses are needed to produce the same response* (Fig. 16-1). In the case of Mr. B, the fact that he never experienced the same high as when he first started taking heroin is a manifestation of the development of tolerance. It should be noted that the drug toxicity profile and the drug lethality profile do not always shift in the same way or to the same degree as the "therapeutic" profile, so that repeated administrations may decrease the therapeutic index of the drug. Thus, it is likely that J.B. developed less tolerance to the side effects of constipation and pupillary constriction than he did to the heroin "high." For the same reason, he was at greater risk of a lethal overdose as his heroin requirement increased. The opposite effect, termed **sensitization** (also called **inverse tolerance**), refers to a shift of the dose–response curve to the left, so that repeated administrations of a drug result in a greater effect of a given dose or require a lower dose to achieve the same effect. Interestingly, tolerance and sensitization to a drug may occur concurrently. The mechanisms that underlie tolerance and sensitization have been the subject of significant research, as described below.

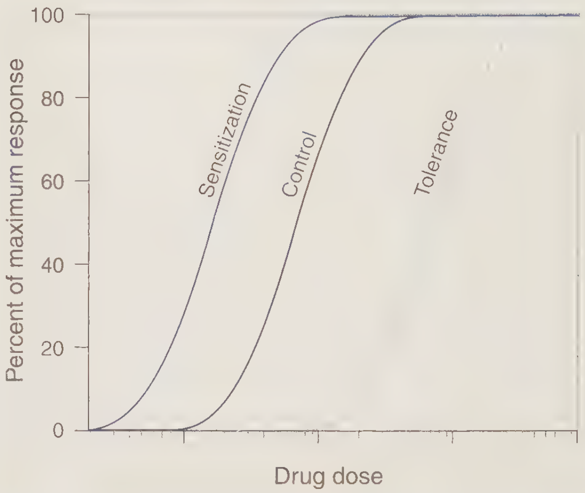


**Figure 16-1. Effects of Tolerance and Sensitization on the Dose–Response Curve.** The first administration of a drug produces a certain dose–response curve (black). Repeated administrations of the drug can lead to the development of tolerance, in which the dose–response curve shifts to the right (light blue). A higher dose is required to produce the same effect, and it is not always possible to achieve the same maximal effect (dashed portion of curve). Sensitization to the effects of a drug means that the dose–response curve shifts to the left (dark blue), and a lower dose is needed to elicit the same response. In addition to the illustrated changes in the apparent potency of the drug, tolerance and sensitization can also be associated with changes in the maximal response produced by the drug.

**Physical dependence** (or **physiologic dependence**) refers to the adverse physical symptoms and signs that result from the absence of a drug. These symptoms are termed **withdrawal** symptoms. In J.B.'s case, his initial symptoms in the emergency room were caused by heroin withdrawal, and his need to continue taking heroin to prevent those symptoms indicated a physical dependence. Physical dependence results from many of the same mechanisms that produce tolerance. As in the case of tolerance, homeostatic set-points are altered to compensate for the presence of the drug. If the drug is withdrawn, the altered set-points produce effects opposite to those manifested in the presence of the drug. For example, abrupt withdrawal from an opioid analgesic produces hypersensitivity to painful stimuli (as well as other symptoms), while abrupt withdrawal from a barbiturate sedative/hypnotic produces insomnia, anxiety, and agitation (among other symptoms).

**Psychologic dependence** is a more complex phenomenon that can occur even with drugs that do not produce tolerance and physical dependence. Psychologic dependence occurs when a drug affects the **reward system** of the brain. The resulting pleasurable sensations cause the user to want to continue taking the drug. When the drug is withheld, adaptations in the brain reward system manifest as dysphoria and drug craving, as Mr. B experienced. Thus, both physical and psychologic dependence result from alterations in homeostatic processes, but these processes occur in different regions of the brain.

Addiction is the most difficult of these terms to define. Perhaps for this reason, the American Psychiatric Association has chosen to use the term "drug dependence" in its current Diagnostic and Statistical Manual (DSM-IV) (Box 16-1), although this term can lead to confusion with the definitions of dependence given above. As defined here, **addiction** refers to *compulsive drug-using (and drug-seeking) behavior that interferes with normal functioning and causes the addict to continue using the drug despite increasingly damaging consequences.* Because Mr. B felt that he was "trapped" by the drug and was compelled to continue using, it is likely that he was addicted to heroin. Addiction may coexist with tolerance and dependence, but the presence of the latter does not necessarily imply addiction. For example, a patient administered an opioid for chronic pain will likely develop tolerance to the drug and require larger doses over time. The patient has not necessarily become addicted to the opioid, however, and it will likely be possible to taper and eventually eliminate the analgesic once the pain abates. In this case, the patient is considered to be tolerant and dependent to the opioid, but not addicted. Tolerance and dependence are normal physiologic adaptations to the continued use of a drug, whereas addiction represents a maladaptive state. A more detailed examination of the mechanisms and factors that affect the likelihood of addiction can be found below.

### BOX 16-1. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Criteria for Substance Dependence (Addiction)

A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:

1. Tolerance, as defined by either of the following:
    a. a need for markedly increased amounts of the substance to achieve intoxication or the desired effect;
    b. markedly diminished effect with continued use of the same amount of substance.
2. Withdrawal, as manifested by either of the following:
    a. the characteristic withdrawal syndrome for the substance (as defined by the APA criteria for withdrawal for a specific substance);
    b. The same (or closely related) substance is taken to relieve or avoid withdrawal symptoms.
3. The substance is often taken in larger amounts or over a longer period than was intended.
4. There is a persistent desire or unsuccessful efforts to cut down or control substance use.
5. A great deal of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects.
6. Important social, occupational, or recreational activities are given up or reduced because of substance use.

The substance use is continued despite knowledge of having a persistent or recurrent physical or psychologic problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption).

# MECHANISMS OF TOLERANCE, DEPENDENCE, AND ADDICTION

## MECHANISMS OF TOLERANCE

As discussed above, tolerance results when repeated administration of a drug results in a shift of the drug's dose–response curve to the right, so that a larger dose (concentration) of the drug is required to produce the same effect. There are multiple mechanisms by which a drug can cause tolerance. **Innate tolerance** refers to interindividual variations in sensitivity to the drug that are present before its first administration. These variations in sensitivity can arise from polymorphisms in the genes for the drug's receptor or the genes that affect the drug's absorption, metabolism, or excretion. As with any multifactorial trait, genetic variability is likely modified by environment. Innate tolerance is not, strictly speaking, a mechanism of drug tolerance as defined above, but it is nonetheless important to recognize that different individuals may have different dose–response curves to the same drug.

Tolerance that develops over time is termed **acquired tolerance.** Three classes of mechanisms govern the development of acquired tolerance: pharmacokinetic, pharmacodynamic, and learned. **Pharmacokinetic tolerance** develops when the ability to metabolize or excrete the drug increases over time. Increased metabolism is most commonly attributable to the induced synthesis of metabolic enzymes such as the cytochrome P450s (see Chapter 4, Drug Metabolism). In such cases, pharmacokinetic tolerance results in a lower plasma drug concentration for any given dose of drug (Fig. 16-2).

**Pharmacodynamic tolerance,** the most important mechanism of tolerance, results from changes in the drug–receptor interaction. These changes may involve a decrease in the number of receptors or a change in the signal transduction pathway (Fig. 16-3). In the short term, changes in receptor number or binding affinity can be caused by inactivation of receptors (e.g., through phosphorylation), internalization, and degradation of cell surface receptors, or other mechanisms. For example, a kinase present in the cytoplasm may phosphorylate (and, thereby, inactivate) only the agonist-bound form of the receptor. For some metabotropic G protein-

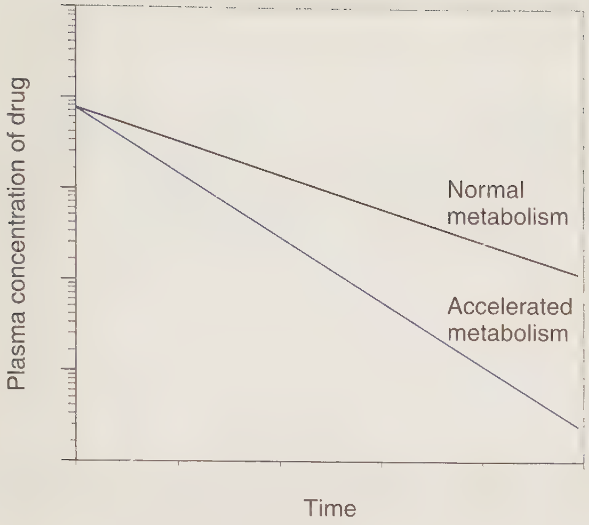


**Figure 16-2. Induction of Tolerance by Increased Metabolism of Drug.** Upon initial administration, a drug that is metabolized by hepatic microsomal enzymes exhibits first-order elimination kinetics (black curve). Upon repeated administrations of the drug, induction of these enzymes causes accelerated metabolism of the drug and a decreased plasma half-life (blue curve). Because the drug is eliminated more rapidly, a higher dose of drug is required to elicit the same response.

coupled receptors, short-term adaptations can include mechanisms that interfere with the coupling between G protein and receptor, for example, by phosphorylation of the receptor or the G protein subunits. Alteration in the conductance of a downstream ion channel by receptor-mediated phosphorylation provides yet another mechanism of tolerance.

In the long term, changes in the number of receptors or other signaling molecules are typically caused by regulation of the genes encoding these proteins. For example, the cellular effects of opioids are mediated through a G protein-coupled metabotropic receptor. Among other effects, opioid binding to the receptor inhibits the activity of adenylyl cyclase (see Chapter 15, Pharmacology of Analgesia). Short-term administration of an opioid such as morphine, therefore, causes a decrease in cellular cAMP. With continued administration of the drug, however, downstream activation of a transcription factor(s) increases the cell's transcription of the adenylyl cyclase gene and, thereby, reduces the cellular response to a given dose (concentration) of morphine (Fig. 16-4). Even longer-lasting effects are postulated to result from certain mechanisms that underlie long-term memory. For example, long-term changes in the pattern of gene expression can alter the expression of AMPA glutamate receptors (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission). In addition, long-lasting cellular adaptations caused by the release of neurotrophic factors may produce persistent adaptations to drug use by modifying existing synapses and creating new synapses, thus effectively "rewiring" the brain. Such long-lasting molecular and cellular adaptations are likely to explain the cravings and relapses that can occur long after drug use has been discontinued, as in the case of J.B.

Another form of tolerance is termed **learned tolerance.** In learned tolerance, a drug produces compensatory changes that are unrelated to its action. The most common mechanism of learned tolerance is behavioral tolerance, in which a person learns to alter his or her behavior to hide the effects of the drug. For example, someone who has been repeatedly intoxicated with alcohol can learn to hide the symptoms of intoxication, such as slurred speech and lack of coordination, and, thus, appear less intoxicated. **Conditioned tolerance** (a type of learned tolerance) occurs when environmental cues associated with exposure to a drug induce pre-emptive compensatory changes. This mechanism of conditioning is an unconscious phenomenon. For example, seeing paraphernalia associated with use of a drug such as cocaine (which produces tachycardia) would elicit a preemptive bradycardia.

## MECHANISMS OF PHYSICAL DEPENDENCE

Physical dependence is a phenomenon that is usually associated with tolerance and that typically results from mechanisms similar to those that produce pharmacodynamic tolerance. Although tolerance refers to the requirement for increasing amounts of a drug to produce the same effect, physical dependence is the need for the drug to be present in the system to maintain normal functioning. If the drug is not present in the system, the adaptations that produced the tolerance are unmasked. *The hallmark of physical dependence is the manifestation of withdrawal symptoms in the absence of the drug.*

As with tolerance, dependence can result from changes in cellular signaling pathways. For example, a drug that produces pharmacodynamic tolerance by upregulating the cAMP pathway also produces dependence because the abrupt withdrawal of the drug allows the upregulated adenylyl cyclase to effect a "supranormal" response. Conversely, a drug that decreases receptor number produces dependence because withdrawal of the drug leaves the downregulated receptors understimulated. These effects are often visible because the receptor has an endogenous agonist whose interaction with the receptor is a part of normal physiologic processes. When a second messenger system is upregulated or a cell surface receptor is downregulated, a "normal" amount of endogenous agonist can effect a supranormal or a subnormal response, respectively. Thus, tolerance and physical dependence arise from similar mechanisms, although the fact that it is possible to have dependence without tolerance and vice versa illustrates our incomplete understanding of these phenomena.

## MECHANISMS OF PSYCHOLOGIC DEPENDENCE

Although the mechanisms of physical dependence are relatively well characterized, the causes of psychologic dependence are still controversial despite much work in this area over the past 50 years.

In the 1950s, Olds and Milner implanted electrodes in various regions of the rat brain to determine which neuroanatomic areas mediate rewarded behavior. The rats were allowed to perform an act (such as pressing a lever) that resulted in a short pulse of nondestructive stimulation at the site of the electrode. The **medial forebrain bundles** and the **ventral tegmental area** (VTA) in the midbrain were found to be particularly effective brain reward sites. A subset of dopaminergic neurons projects directly from the ventral tegmental area to the **nucleus**

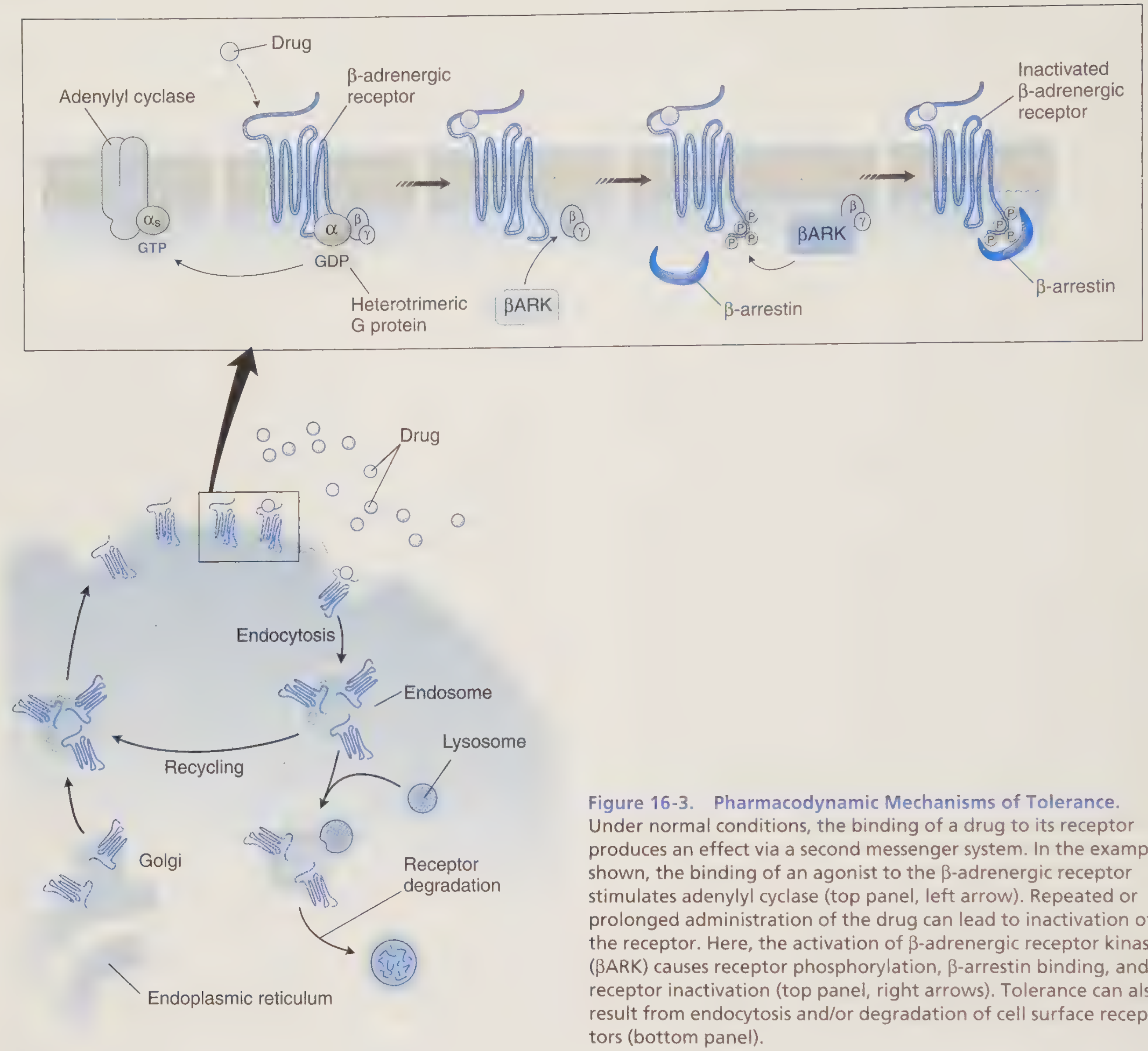


**Figure 16-3. Pharmacodynamic Mechanisms of Tolerance.** Under normal conditions, the binding of a drug to its receptor produces an effect via a second messenger system. In the example shown, the binding of an agonist to the β-adrenergic receptor stimulates adenylyl cyclase (top panel, left arrow). Repeated or prolonged administration of the drug can lead to inactivation of the receptor. Here, the activation of β-adrenergic receptor kinase (βARK) causes receptor phosphorylation, β-arrestin binding, and receptor inactivation (top panel, right arrows). Tolerance can also result from endocytosis and/or degradation of cell surface receptors (bottom panel).

**accumbens** (NAc) via the medial forebrain bundle. It is believed that these neurons are crucial to the brain reward pathway, because severing this pathway, or blocking dopamine receptors in the nucleus accumbens with a dopamine receptor antagonist (such as haloperidol; see Chapter 11, Pharmacology of Dopaminergic Neurotransmission), decreases the rewarding effects of stimulating the ventral tegmental area. Moreover, release of dopamine in the nucleus accumbens can be detected using techniques such as microdialysis. These measurements show that dopaminergic synapses of the nucleus accumbens are active during stimulation of the brain reward pathway and that dopamine in the nucleus accumbens is necessary for reward. Drugs capable of causing psychologic dependence induce the greatest dependence when administered directly to the ventral tegmental area, the nucleus accumbens, or the cortical or subcortical areas that innervate these two areas.

The natural role of the brain reward pathway has been explored in some detail. It is known that dopamine is released in the nucleus accumbens of rats while the animals are eating after a fast or eating particularly palatable food. (Thus, the assertion that chocolate is "addictive" has a mechanistic rationale, even though most "chocoholics" would not meet the DSM-IV criteria in Box 16-1.) The reward pathway is also activated in rats during mating behaviors and certain social interactions. The nucleus accumbens receives inputs from a number of brain regions in addition to the dopaminergic ventral tegmental area. Pathways have been found from several areas of the cortex, thalamus, and amygdala, and from serotonergic nuclei.

Although the discovery that drugs of abuse activate the brain reward pathway may seem to offer an easy explanation for psychologic dependence (i.e., that administration of a drug is associated with reward), recent literature has suggested that psychologic dependence is more complicated. It is likely that many of the molecular mechanisms that mediate physical dependence, such as the upregulation of cAMP signaling pathways, are also important in psychologic dependence. In this formulation, the distinction between physical and psychologic dependence arises not because different molecular mechanisms are operative, but because drug-induced changes affect neurons having different functions. For example, a drug could be acutely euphorigenic because it activates the dopaminergic brain reward pathway, but the euphoria could be followed by a period of dysphoria. If the brain reward pathway undergoes adaptations upon repeated administration of the drug, then these adaptations would be unmasked during withdrawal from the drug, resulting in a state of psychologic dependence.

## MECHANISMS OF ADDICTION

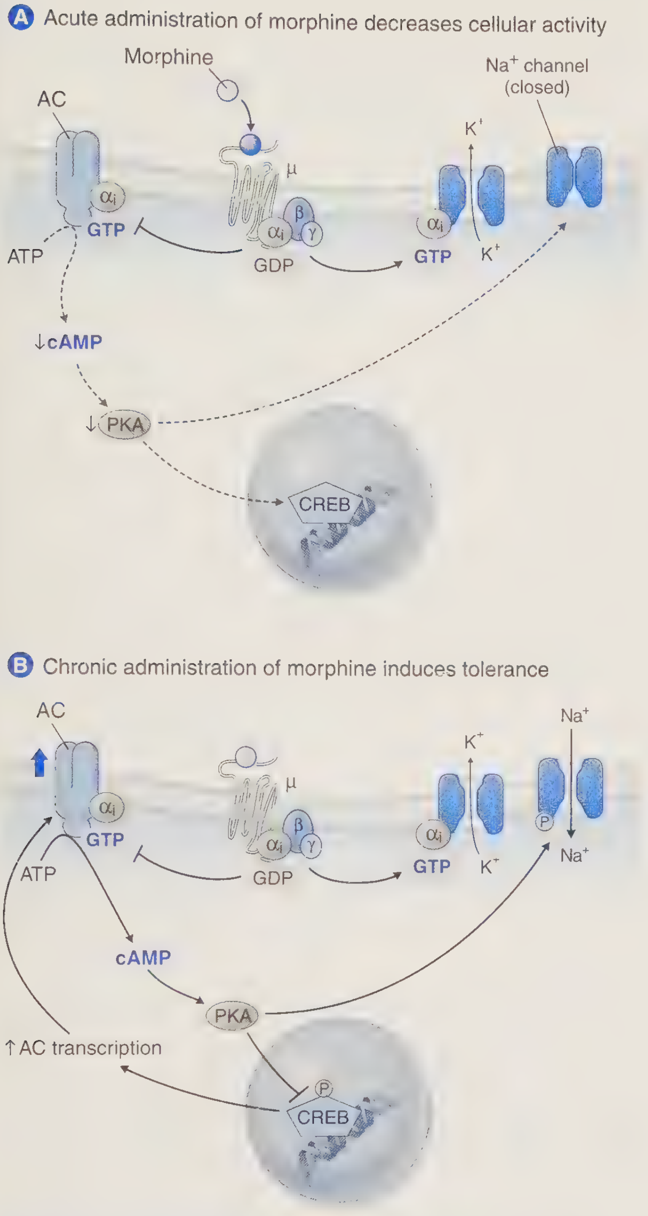
Addiction was originally thought to depend primarily on the physical or psychologic effects of withdrawal. Because withdrawal is an aversive event, the need to maintain blood levels of the drug was thought to be a compelling reason for continued use of the drug. For a number of reasons, however, physical dependence has in recent years been de-emphasized as a cause of addiction. First, the effects of addiction are felt long after the physical symptoms of withdrawal have abated. Years after an addict has discontinued use of a substance, the addict can experience intense **cravings** for the drug (i.e., an intense preoccupation with obtaining the drug), suggesting that a form of psychologic dependence may be long-lasting. Thus, addicts are prone to **relapse,** even after years of sobriety. The likelihood of relapse is especially strong in situations where patients simultaneously encounter both stress and the context in which the drug was previously used. In part, this increased likelihood of relapse may result from an interplay, between reward circuitry and memory circuitry in the brain, that exists under normal circumstances to assign emotional value to certain memories.

Second, physical dependence is unlikely to be the primary mechanism of addiction for such drugs as cocaine and amphetamines, which cause few symptoms of physical dependence, but are, nonetheless, highly addictive. Finally, most interviews with addicts in recovery suggest that addicts arrange their priorities to obtain more drug, not for fear of the physical symptoms associated with withdrawal, but because they are constantly striving for the euphorigenic experience of the high. These observations suggest that chronic drug use causes a long-lasting change in the underlying brain reward system and/or in memory systems related to the reward system.

### Variables Affecting the Development of Addiction

The development of addiction is dependent on a number of variables, including the nature of the drug, genetic and other traits of the drug user, and environmental variables.

As noted above, the ability of a drug to produce psychologic dependence is strongly correlated with its ability to cause addiction. Pharmacokinetic properties of the drug also affect its propensity to cause addiction. In general, short-acting drugs are more addictive than long-acting drugs because the clearance of a long-acting drug results in a slow decrease in drug concentration over time, preventing acute withdrawal. In addition, the more rapid the rise in drug concentration at the target neuron, the greater the potential for addiction. Direct injection or rapid absorption of drug through a large surface area (e.g., the lungs via smoking) is more highly addictive than absorption through the intestinal or nasal mucosa. The importance of this effect is demonstrated by the potential for abuse


**A** Acute administration of morphine decreases cellular activity

**B** Chronic administration of morphine induces tolerance

**Figure 16-4. Induction of Tolerance to Morphine. A.** The μ-opioid receptor is coupled to a G protein that activates potassium channels and inhibits adenylyl cyclase (AC), resulting in membrane hyperpolarization and in decreased production of cAMP. Because cAMP activates protein kinase A (PKA), which in turn regulates the threshold of the voltage-gated sodium channel, the decreased cAMP levels indirectly decrease sodium channel conductance. Decreased cAMP also decreases the activation of the transcription factor cAMP response element-binding protein (CREB), which regulates the level of AC expression. **B.** Chronic administration of morphine up-regulates CREB, which stimulates the transcription of adenylyl cyclase, which in turn restores cAMP production toward normal levels. The increased cAMP stimulates PKA, which phosphorylates (and thereby activates) both CREB and the voltage-gated sodium channel. Up-regulation of the cAMP pathway, therefore, counteracts the acute effects of the drug, resulting in tolerance.

of various forms of cocaine (Fig. 16-5). The use of coca leaves as a chew or in teas is widely practiced in the Andean mountains and has a relatively low potential for addiction because of the slow rate of rise and low peak concentration of drug attained by absorption through the buccal or intestinal mucosa. The rapid absorption of extracted cocaine hydrochloride through the nasal mucosa is significantly more addictive. The most addictive forms of cocaine are intravenous injections and inhalation of smoked freebase (crack cocaine), both of which result in a very rapid rise in plasma concentration and a high peak concentration of drug.

Different people react differently to drugs. Some individuals use a drug once and never use it again; others use a drug repeatedly in moderate amounts without developing an addiction; in others, the first use of a drug produces such an intense euphoric feeling that the likelihood of addiction is high. The factors discussed above (i.e., the chemistry of the molecule and its site of action; the peak and rate of rise of drug concentration; the context in which the drug is used; previous experiences; and the ease with which the experience can be repeated) all interact with the genetics, personality, and environment of the individual to affect the probability of drug addiction.

**Genetic influences** have been best studied in individuals with alcohol abuse. There is evidence for an influence of genetics on the susceptibility to alcoholism, although even identical twins are not completely concordant. That is, the likelihood that the identical twin of an alcoholic will also be an alcoholic is greater than the likelihood among fraternal twins, but is less than 100%. The observed concordance between identical twins may be a result of innate tolerance to alcohol. One study found that, compared to subjects who are

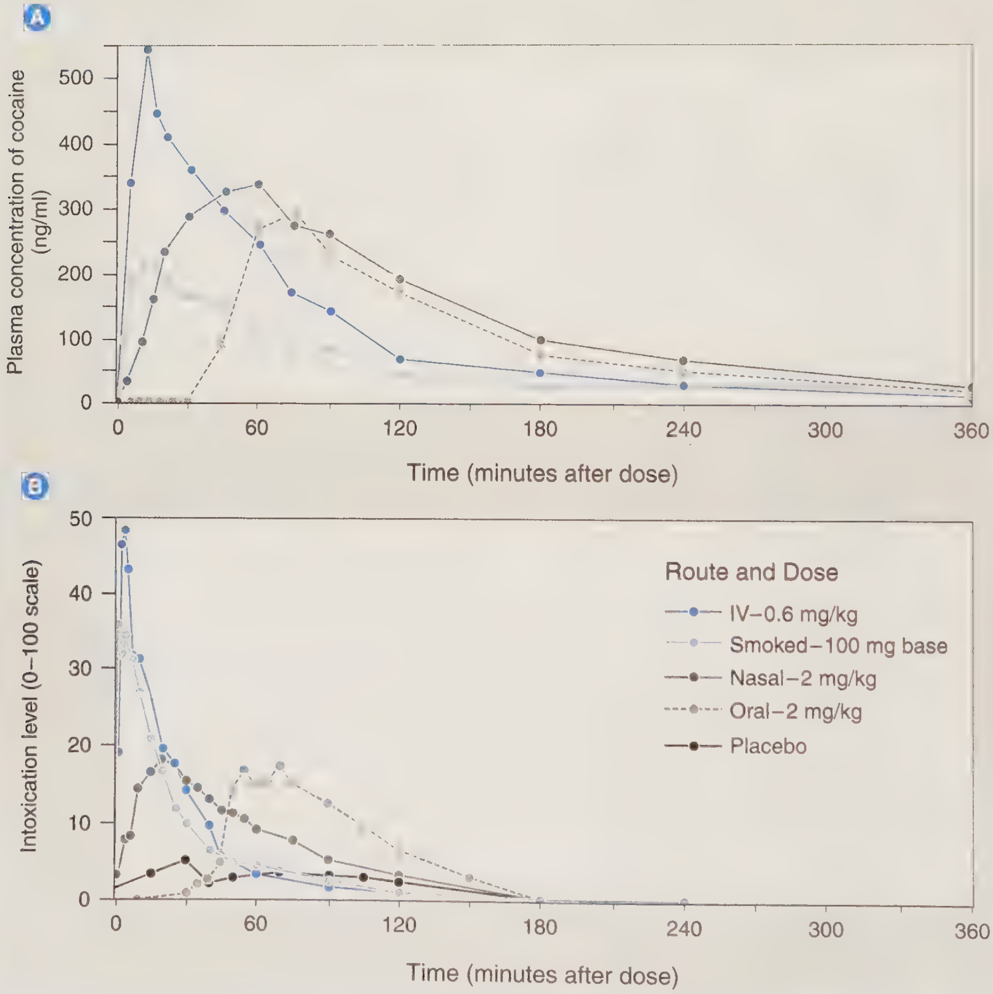


**Figure 16-5. Plasma Cocaine Concentrations and Levels of Intoxication as a Function of Route of Administration of the Drug.** The pharmacokinetics (**A**) and pharmacodynamics (**B**) of cocaine are highly dependent on the route of administration of the drug. Intravenous (IV) cocaine and smoked free-base cocaine are associated with very rapid attainment of the peak plasma drug concentration (**A**) and with high levels of intoxication (**B**). In contrast, the nasal and oral routes of administration are associated with a slower rise in plasma drug concentration (**A**) and lower levels of intoxication (**B**). Because of the very rapid rise in plasma drug concentration and very high intoxication levels, smoked and intravenous cocaine carry a higher risk of addiction than cocaine taken nasally or orally.

less sensitive to the intoxicating effects of alcohol, subjects who are more sensitive are less likely to become alcoholics. Alternatively, polymorphisms in the dopamine D2 receptor gene may be correlated with increased susceptibility to alcohol abuse. Two such polymorphisms are expressed disproportionately in alcoholics and other substance abusers, as compared to control individuals.

**Personality differences** may contribute to the risk of developing drug abuse. Psychologists describe risk-taking personalities and risk-averse personalities, and there is some evidence that risk takers are more likely to become addicted to drugs than are risk-averse individuals. Finally, other diseases, especially psychiatric illnesses, are associated with a predilection to drug abuse; drug use in this case may be an attempt to self-medicate. Alcohol, for example, is widely used as a self-medication for anxiety (i.e., as an anxiolytic), and amphetamines may be used to self-medicate for attention deficit disorder. However, it is often difficult to determine whether such illnesses are the cause of drug abuse or its effect because the effects of the drugs themselves, as well as drug withdrawal and dependence, can unmask psychiatric illness in a previously compensated individual.

Finally, **environmental variables** have a strong influence on the development of addiction. For example, societal attitudes toward drug-taking behavior often influence the likelihood that a drug will be taken in the first place. The availability and cost of the drug may also be affected by its legal and tax status. The availability of other, nondrug alternatives may be a key factor in determining the likelihood of drug use and addiction. Given the important role that context plays in the development of addiction, it is clear that environmental variables interact strongly with the chemical actions of drugs.

# DRUGS OF ABUSE

Having focused on the mechanisms by which drugs cause dependence and addiction, the discussion now turns to a number of commonly used classes of drugs. A number of drugs with the potential to cause dependence and addiction are commonly prescribed therapeutics (e.g., opioids, barbiturates, benzodiazepines), and their mechanisms of action have been discussed in detail in previous chapters. Other commonly abused drugs (e.g., heroin) are not generally prescribed, but act via the same mechanisms as their prescription counterparts. Yet other drugs of abuse (e.g., phencyclidine) act on proteins that are not currently the target of commonly prescribed drugs. These agents, however, affect many of the same neurotransmitter systems that have been described in previous chapters. Finally, some drugs (e.g., cannabis) affect receptors that have not been discussed in this book, because those receptors are not yet targets for therapeutic intervention. Nonetheless, the mechanisms of action of these drugs have many parallels to receptors and systems that have been described in earlier chapters.

## COMMONLY PRESCRIBED THERAPEUTICS

Many commonly prescribed therapeutics can cause dependence and, occasionally, addiction. This category includes three important classes of drugs: **opioids, benzodiazepines,** and **barbiturates.** The mechanisms of action and general pharmacology of opioids are described in Chapter 15, and those of barbiturates and benzodiazepines are described in Chapter 10.

### Opioids

The mechanism by which opioids cause physical dependence has been well studied and is explained in detail above. As the case of Mr. B illustrates, chronic opioid use can produce a significant probability of relapse that persists long after the physical symptoms of dependence have abated. There seems to be two pathways by which opioids interact with the brain reward system (Fig. 16-6). One site of action lies in the ventral tegmental area, where GABAergic interneurons tonically inhibit the dopaminergic neurons responsible for activating the brain reward pathway in the nucleus accumbens. These GABAergic interneurons can be inhibited by endogenous enkephalins, which bind to μ-opioid receptors on the GABAergic terminals. Because exogenous opioids, such as morphine, also bind to and activate μ-opioid receptors (see Chapter 15), it follows that an exogenously administered opioid could activate the brain reward pathway by disinhibiting dopaminergic neurons in the ventral tegmental area. The second pathway, which has not been studied as well, is localized in the nucleus accumbens. Opioids acting in this region may inhibit GABAergic neurons that project back to the ventral tegmental area, perhaps as part of an inhibitory feedback loop. The relative importance of these two pathways is still being debated.

Although all opioids have the potential to cause tolerance and physical dependence, certain opioids are more likely than others to cause addiction. As may be expected, the opioids associated with the fastest rise in brain concentrations of drug have the highest likelihood for abuse. Likewise, abuse of the drug oxycodone (sold as OxyContin®), which is commonly prescribed for moderate or severe pain, has received much recent publicity because of allegations of misuse and cases of addiction. One reason for the highly addictive nature of oxycodone is that the oral tablets can be broken up, dissolved, and injected. This form of administration results in a much more rapid rise in plasma (and hence brain) concentrations of drug, a more intense feeling of euphoria, and a greater addiction potential as compared to the normally prescribed, slow-release oral form of the drug.

### Benzodiazepines and Barbiturates

Benzodiazepines and barbiturates can also cause both physical dependence and addiction, although addiction is rare. Because benzodiazepines and barbiturates increase the efficiency of GABAergic pathways, chronic use of these drugs can induce down-regulation of these pathways by a compensatory mechanism. One possible mechanism of down-regulation is the uncoupling of the benzodiazepine site from the GABA site on $GABA_A$ receptors (see Chapter 10). Thus, the binding of benzodiazepines to $GABA_A$ would remain unchanged, but the drug would have little or no potentiating effect on the binding of GABA to the receptor. Down-regulation of inhibitory GABAergic pathways would be expected to leave the brain “underinhibited,” increasing the possibility of seizures (see Chapter 13), delirium, and occasionally death upon abrupt withdrawal of the benzodiazepine or barbiturate. Underinhi-

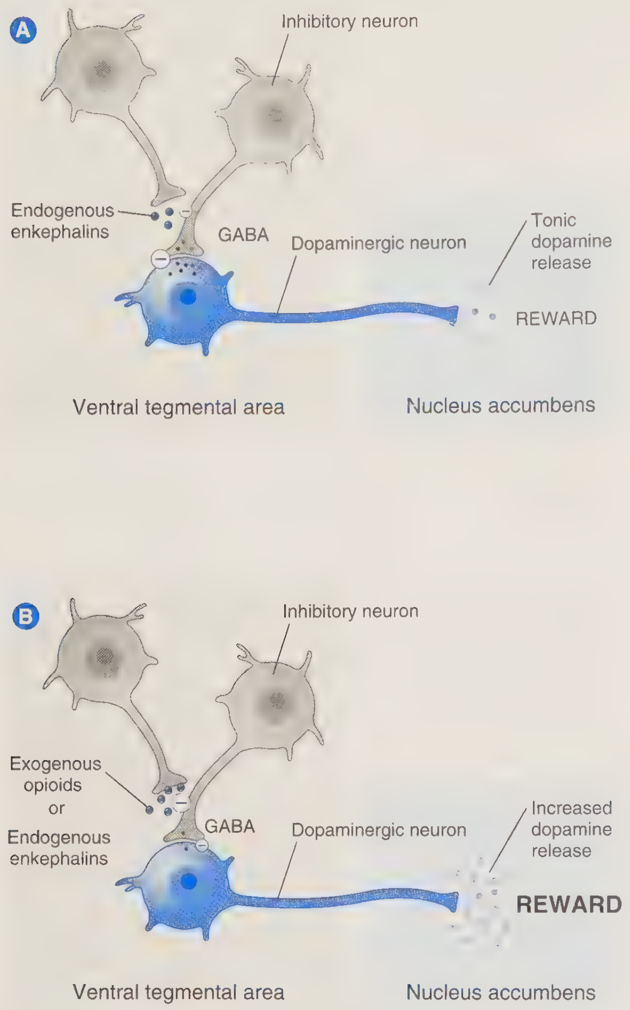


**Figure 16-6. Role of Opioids in the Brain Reward Pathway.** **A.** GABAergic neurons tonically inhibit the dopaminergic neurons that arise in the ventral tegmental area and are responsible for reward. These GABAergic neurons can be inhibited by endogenous enkephalins, which locally modulate the release of neurotransmitter at the GABAergic nerve terminal. **B.** Administration of exogenous opioids results in decreased GABA release and disinhibition of the dopaminergic reward neurons. The increased release of dopamine in the nucleus accumbens signals a strong reward.

bition of pathways involving central sympathetic activity can lead to such physical symptoms as anxiety, sleep disturbance, and dizziness. Similarly, underinhibition of pathways governing anxiety, fear, confusion, and panic can cause psychologic dependence and lead to addiction. Thus, the potential consequences of benzodiazepine and barbiturate withdrawal should not be ignored.

It is important to note that most prescribed therapeutics cause addiction only rarely, and that the potential for addiction should not deter physicians or other health professionals from prescribing a therapeutic for legitimate medical purposes. Unfortunately, opioids are often underprescribed for the treatment of pain because tolerance (manifested as a request for higher and higher doses of drug) is mistaken for addiction. This condition is often referred to as **pseudoaddiction.** Because tolerance is an expected effect of the drug, physicians should be prepared to increase the dose, if necessary, to control the patient's pain. Because of the high potential for withdrawal symptoms upon discontinuation of therapy, physicians should also be careful to taper the dose of such a medication that has been prescribed for an extended period and to explain to the patient the rationale for the taper.

Opioids, benzodiazepines, and barbiturates can be misused. For example, a patient in possession of medication that is left over from a previous prescription may use it inappropriately for another indication. Similarly, a patient may "share" medication with someone else outside a physician's supervision. For a variety of reasons, especially an inability to obtain more of the drug, this type of drug misuse rarely leads to addiction. In contrast, some patients may exhibit drug-seeking behavior and resort to forging prescriptions or obtaining prescriptions from multiple physicians, especially after suboptimal management of the underlying condition (e.g., failing to taper slowly a drug that has resulted in dependence). In some cases, a "black market" for the drug may develop (note the example of oxycodone cited above). It bears repeating, however, that, although cases of addiction receive much publicity, undermedication of pain is much more common than addiction to pain medication.

Another serious concern is the misuse of prescription opioids (or, less commonly, benzodiazepines or barbiturates) by health professionals. For at least two reasons, health professionals who misuse prescription medications are at greater risk for developing addiction. First, they have more ready access to prescription medication. Second, they may believe that, because they understand a drug's effects, they will be able to control its use more easily.

## DRUGS RELATED TO THERAPEUTICS

The second category of commonly abused drugs consists of agents that bind to the same receptors as commonly prescribed therapeutics, but that are not themselves used as therapeutic agents. For example, **heroin** binds to the same receptor as **morphine. Alcohol,** which binds to the $\text{GABA}_A$ receptor, and **nicotine,** which binds to the nicotinic acetylcholine receptor (see Chapter 7, Cholinergic Pharmacology), mimic other GABAergic and cholinergic agonists, respectively. **Cocaine** and **amphetamine** have actions similar to those of other drugs that inhibit monoamine reuptake transporters (see Chapters 8 and 12). For these drugs, it is possible to understand their effects by understanding the effects of their prescribed counterparts.

### Heroin

As with morphine, heroin exerts its effects by binding to the μ-opioid receptor. The difference in the action of the two drugs is primarily because of differences in their pharmacokinetics. The two drugs are close structural analogues (heroin is deacetylated to 6-monoacetylmorphine, and morphine is acetylated to the same compound), but heroin is more hydrophobic than morphine. Because of this property, heroin crosses the blood–brain barrier more rapidly. The more rapid increase in brain concentrations of heroin produces a sharper "high," which explains why heroin is typically preferred over morphine as a drug of abuse. The mechanism by which heroin produces dependence and addiction is identical to that of morphine and the other opioids.

## Alcohol

Alcohol (specifically, ethanol) affects a number of different receptors, including $GABA_A$ receptors, NMDA glutamate receptors (see Chapter 10), and cannabinoid receptors (see below). Although the specific sites of action are unknown, $GABA_A$ channels are believed to mediate the anxiolytic and sedative effects of alcohol, as well as the effects of alcohol on motor coordination, tolerance, dependence, and self-administration. Alcohol increases GABA-mediated chloride conductance and enhances hyperpolarization of the neuron (see Chapter 10). Its mechanisms of dependence and addiction are likely to be similar to those of other drugs affecting the GABA neurotransmission system.

Evidence also points to a role for NMDA receptors in the development of tolerance and dependence to alcohol, and NMDA receptors may have a role in the alcohol withdrawal syndrome. Specifically, alcohol inhibits subtypes of NMDA receptors that seem to be capable of long-term potentiation. Thus, although GABA receptors play a vital role in mediating the effects of alcohol, the ability of alcohol to interact with a number of different receptor types suggests that our understanding of its mechanisms of action remains incomplete.

## Nicotine

Nicotine directly activates nicotinic acetylcholine receptors (see Chapter 7) that are located centrally, peripherally, and at the neuromuscular junction. Centrally, nicotine produces a strong dependence, as the craving for cigarettes is directly tied to decreases in the plasma levels of nicotine. Cholinergic neurons arising from the **laterodorsal tegmental area** (near the border of the midbrain and pons) activate nicotinic and muscarinic acetylcholine receptors on dopaminergic neurons in the ventral tegmental area; stimulation of these nicotinic receptors by nicotine activates the dopaminergic brain reward pathway (Fig. 16-7). This strong and direct effect on the reward pathway explains the high addiction potential of nicotine.

## Cocaine and Amphetamine

By blocking or reversing the direction of the neurotransmitter transporters that mediate reuptake of the monoamines dopamine, norepinephrine, and serotonin into presynaptic terminals, **cocaine** and **amphetamine** potentiate dopaminergic, adrenergic, and serotonergic neurotransmission. Cocaine is most potent at blocking the dopamine transporter (DAT), although higher concentrations block the serotonin and norepinephrine transporters (5HTT and NET, respectively) as well. Recall that the tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) function in a similar manner, blocking reuptake of norepinephrine and serotonin (TCAs) or serotonin alone (SSRIs) into presynaptic neurons (see Chapter 12). Amphetamine reverses the direction of all three monamine transporters, although this drug is most selective for the norepinephrine transporter. Amphetamine also releases vesicular transmitter stores into the cytoplasm; these combined actions cause the catecholamine neurotransmitter to be transported into, rather than out of, the synaptic cleft. By these actions, cocaine and amphetamine increase the concentration of monoamine neurotransmitters in the synaptic cleft, potentiating neurotransmission (Fig. 16-8).

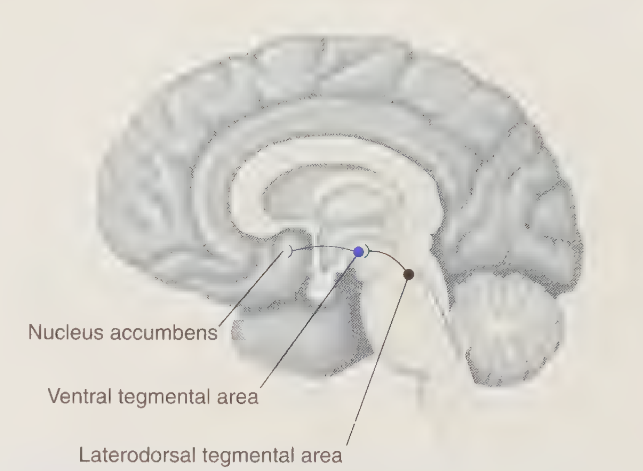


**Figure 16-7. Role of Cholinergic Neurotransmission in the Brain Reward Pathway.** Nicotinic neurons (black) arising in the laterodorsal tegmental area (DTA) activate dopaminergic neurons (blue) in the ventral tegmental area (VTA). These neurons, which make up the brain reward pathway, release dopamine in the nucleus accumbens (NAc).

Although cocaine and amphetamine act on monoamine neurons throughout the body, it is the action of these drugs on neurons in two major centers in the brain that likely governs their potential for abuse (Fig. 16-9). The first set of neurons, in the **locus ceruleus** in the pons, sends ascending adrenergic projections throughout the hypothalamus, thalamus, cerebral cortex and cerebellum, and descending projections to the medulla and spinal cord. These projections maintain alertness and responsiveness to unexpected stimuli (see Chapter 8, Adrenergic Pharmacology). Thus, such drugs as cocaine and amphetamine, which potentiate the actions of norepinephrine by inhibiting neurotransmitter reuptake, produce enhanced arousal and vigilance. For this reason, cocaine and amphetamine are considered to be psychostimulants.

The second major site at which cocaine and amphetamine act is on midbrain dopaminergic neurons whose axons terminate in the nucleus accumbens, striatum, and cortex. As discussed above, the dopaminergic terminals in the nucleus accumbens are a critical component of the brain's reward pathway.

Given the widespread distribution of monoamine neurons in the CNS, it is not surprising that cocaine and amphetamine have a number of effects in addition to psychostimulation. These drugs can cause paranoia and delusions, effects that may be related to potentiation of neurotransmission in the dopaminergic projections to the cortex, thalamus, and amygdala that may be involved in schizophrenia (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission). Cocaine and amphetamine can also cause involuntary movements through their action on the basal ganglia, in much the same way that the anti-Parkinsonian dopaminergic drugs can cause dyskinesias associated with "on periods" (see Chapter 11).

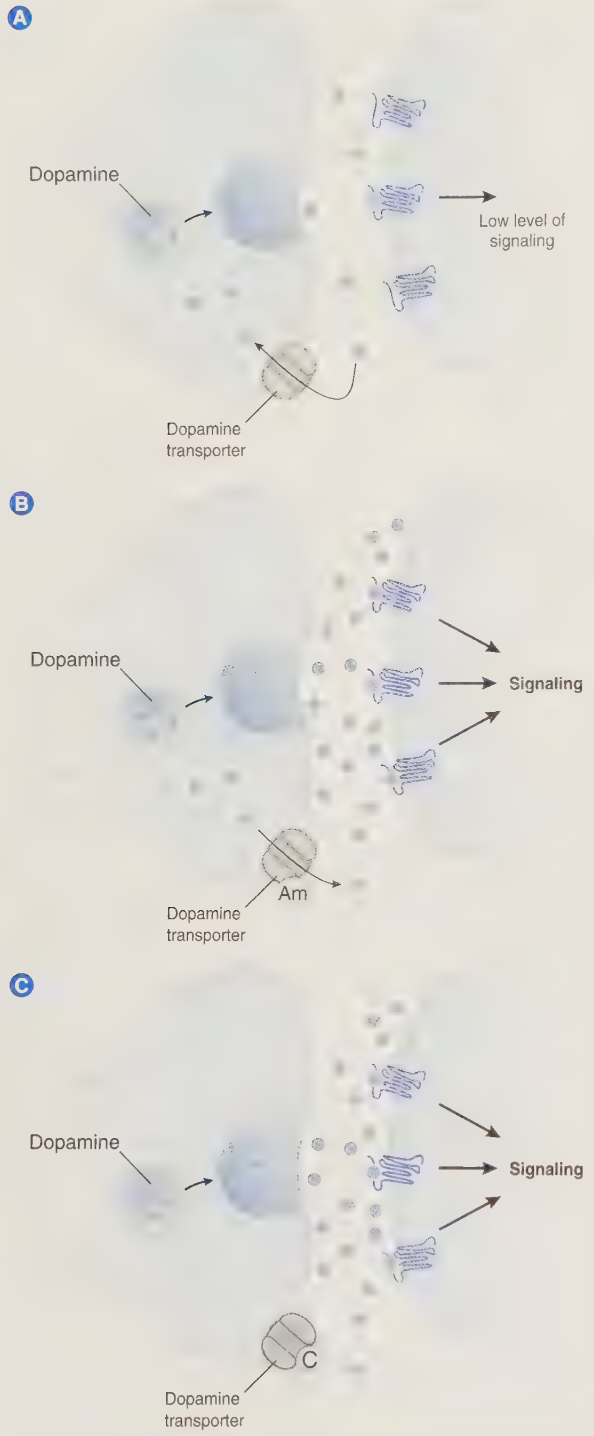


**Figure 16-8. Mechanism of Action of Cocaine and Amphetamine.** **A.** In normal dopaminergic neurotransmission, dopamine released from synaptic vesicles is cleared from the synapse by dopamine reuptake transporters in the presynaptic membrane. **B.** Amphetamine (Am) releases dopamine from synaptic vesicles into the cytosol and reverses the direction of dopamine transport through the dopamine transporter. Together, these actions increase the concentration of dopamine in the synaptic cleft and, thereby, potentiate neurotransmission. **C.** Cocaine (C) potentiates dopaminergic neurotransmission by blocking the dopamine reuptake transporter and, thereby, increasing synaptic dopamine concentration. Cocaine and amphetamine have similar effects at noradrenergic and serotonergic nerve terminals.

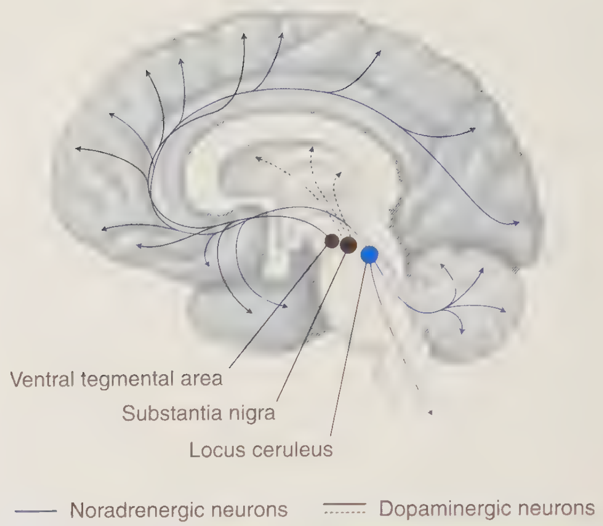


**Figure 16-9. Locus of Action of Cocaine and Amphetamine.** Amphetamine and cocaine act on noradrenergic neurons originating in the locus ceruleus and projecting throughout the cerebral cortex, hypothalamus, cerebellum and spinal cord (blue). Noradrenergic neurons that terminate in the cerebral cortex maintain alertness. Amphetamine and cocaine also act on dopaminergic neurons originating in the ventral tegmental area and projecting to the cerebral cortex, hypothalamus, and nucleus accumbens (black solid lines). Dopaminergic neurons that terminate in the nucleus accumbens are an important component of the brain reward pathway. Other dopaminergic neurons originating in the substantia nigra and projecting to the striatum (black dashed lines) help initiate intended movement.

Cocaine and amphetamine have widespread actions in peripheral tissues because there is an extensive distribution of adrenergic neurons in the peripheral nervous system (see Chapter 8). These potentiators of norepinephrine neurotransmission increase heart rate and blood pressure through the increased action of norepinephrine on β-adrenergic receptors. Cocaine, in particular, can cause vasospasm leading to stroke, myocardial infarction, or aortic dissection.

It was long believed that the psychostimulants do not cause physical dependence. However, cocaine use can be associated with such withdrawal symptoms as bradycardia, sleepiness, and fatigue. Withdrawal from cocaine or amphetamine also causes psychologic symptoms, such as dysphoria and anhedonia (an inability to experience pleasure), that are opposite to the euphoria experienced immediately following administration of the drug. Many of these symptoms are not strictly attributable to withdrawal because they cannot be alleviated by the administration of more cocaine or amphetamine. In fact, symptoms of withdrawal can appear even when psychostimulant levels in the plasma are still high. This is because the drugs cause **tachyphylaxis** (Latin for "rapid protection"), an acute process in which the target tissue becomes less and less responsive to constant concentrations of a drug. In the case of cocaine and amphetamine, tachyphylaxis may be caused by depletion of the neurotransmitter. Because the drugs block presynaptic neurotransmitter reuptake, over time the transmitter diffuses away from the synaptic cleft and neurotransmitter stores in the presynaptic terminal are depleted. This may explain why administering such a dopamine receptor agonist as **bromocriptine** can alleviate the symptoms of withdrawal.

## DRUGS OF ABUSE AFFECTING OTHER RECEPTORS

A number of commonly abused drugs bind to receptors that have yet to be exploited therapeutically. One such drug, **phencyclidine** (PCP), blocks *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. NMDA receptors mediate excitatory synaptic transmission and are involved in synaptic plasticity and memory (see Chapter 10). PCP interferes with these processes, producing such complex effects as anesthesia, delirium, hallucinations, and amnesia.

The drug **methylenedioxymethamphetamine (MDMA)**, known colloquially as ecstasy, has received recent attention because of its increased use and the unfortunate perception that it is a "safe" drug. Although it is chemically related to methamphetamine and does have similar dopaminergic effects, the primary effect of MDMA is on serotonergic neurotransmission. MDMA causes serotonin release into the synaptic cleft, inhibition of serotonin synthesis, and block of serotonin reuptake. Together, these complex actions of MDMA increase serotonin in the synaptic cleft while depleting presynaptic stores of the neruotransmitter. The drug causes a central stimulant effect like cocaine and amphetamine but, unlike the latter drugs, it also has hallucinogenic properties. Like cocaine and amphetamine, MDMA affects the brain reward pathway through dopaminergic stimulation. Finally, MDMA may be neurotoxic to a subpopulation of serotonergic neurons when the drug is administered repeatedly or in large amounts.

**Cannabinoids** are compounds found in cannabis (marijuana). These natural products bind to cannabinoid receptors, which are G protein-coupled receptors whose endogenous ligand is the arachidonic acid derivative anandamide. The two known cannabinoid receptors (CB1 and CB2) are widely distributed in the basal ganglia (including the substantia nigra, pars reticulata, and globus pallidus), the hippocampus and the brainstem, and the effects of cannabis are correspondingly diffuse. Cannabis causes a prompt and generalized "high" characterized by euphoria, laughter, giddiness, and depersonalization. After 1–2 hours, cognitive functions such as memory, reaction time, coordination, and alertness are compromised, and the user has difficulty concentrating. This effect corresponds to a "mellowing" phase, which results in relaxation and even sleep. In rats, the administration of natural and synthetic cannabinoids causes dopamine release in the nucleus accumbens of the brain reward pathway, although the specific pathway involved is not yet known.

Cannabis use induces tolerance; the mechanism of this effect remains to be elucidated. Discontinuation of cannabis is followed by a withdrawal syndrome that includes restlessness, irritability, agitation, insomnia, and nausea. There is evidence that enkephalins are involved in this withdrawal syndrome.

Finally, the widely used stimulant **caffeine** acts by blocking adenosine receptors that are expressed presynaptically on many neurons, including adrenergic neurons. Because activation of the adenosine receptors inhibits norepinephrine release, competitive antagonism of the receptors by caffeine disinhibits norepinephrine release and, thus, acts as a stimulant. Caffeine may also block adenosine receptors on cortical neurons and, thereby, disinhibit these neurons. Caffeine can cause withdrawal effects such as lethargy and a characteristic headache, but addiction, though documented, is rare.

## TREATMENTS FOR ADDICTION AND DEPENDENCE

Treatments for addiction can be divided into two broad approaches, **pharmacologic** and **social.** Traditionally, pharmacologic treatments for addiction have focused on detoxification to relieve the withdrawal symptoms that accompany the cessation of drug use. Pharmacologic agents used to treat addiction are summarized in the Drug Summary Table at the end of this chapter. Although these agents can be very effective in short-term treatment strategies, one of the hallmarks of drug addiction is a high likelihood of relapse, even after years of sobriety. Relapse seems to occur because addiction entails a long-lasting adaptation in brain reward pathways. For this reason, drug abuse is considered a chronic problem, and therapy must include long-term as well as short-term management of the addict. Social treatment has traditionally focused on the long-term, and has generally been more successful than short-term pharmacologic treatment in producing long-lasting abstinence. Moreover, although health care teams typically focus on treatment of individual patients, effective treatment must also address the underlying social causes of drug abuse: unemployment, poverty, racism, sexism, abuse, and other sources of stress.

## DETOXIFICATION

The acute treatment for drug addiction is **detoxification.** The goals of detoxification are to allow blood levels of the drug to fall to near zero and to allow the body to adapt to the absence of drug. Thus, a standard detoxification program consists of a 28-day inpatient rehabilitation protocol that includes supportive medication to suppress withdrawal effects, such as the program completed by J.B.

The manifestations of drug withdrawal depend on the type of drug abused and can range from mild dysphoria to life-threatening convulsions. The most commonly employed strategies for alleviating withdrawal effects are to taper the dose of the drug slowly and/or to use a long-acting drug in the same drug class. For example, a common treatment for nicotine withdrawal is the administration of nicotine via a sustained release transdermal patch or via a chewing gum. The dose is tapered slowly to allow the patient to avoid many of the unpleasant effects of nicotine withdrawal. Another example is the use of **methadone** for the treatment of addiction to an opioid, such as heroin. Methadone is a long-acting opioid that is administered as an oral tablet and, hence, has a much slower uptake than heroin. The combination of the slow onset and long plasma half-life cause the plasma levels of methadone to remain fairly constant, and the drug can be tapered slowly while avoiding the acute effects of opioid withdrawal (Fig. 16-10). Withdrawal symptoms from alcohol and benzodiazepines can be severe, in some cases even life-threatening. In these cases, administration of a long-acting benzodiazepine such as **diazepam** is indicated to prevent withdrawal seizures.

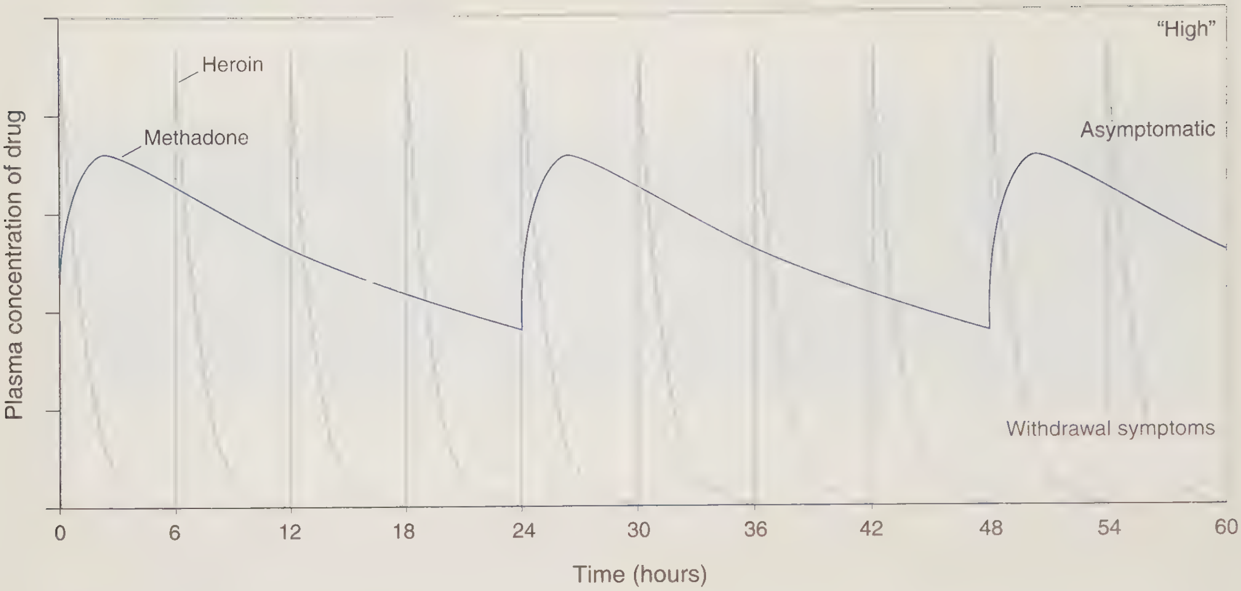


Figure 16-10. Pharmacokinetics and Pharmacodynamics of a Fast-Acting Opioid (Heroin) Compared to a Slow-Acting Opioid (Methadone). The plasma concentration of a fast-acting opioid such as heroin rises rapidly after intravenous administration, generating a "high," but also falls quickly, producing withdrawal symptoms. On the other hand, the plasma concentration of a slow-acting, long half-life drug such as methadone remains in the asymptomatic range for a period of over 24 hours, so that the patient does not experience either the "high" or the withdrawal symptoms. Moreover, because of its long plasma half-life, methadone needs to be administered only once per day.

A newer **rapid-detox** protocol has recently been developed for the treatment of opioid addiction. This protocol involves placing the patient under general anesthesia for up to 24 hours, during which an opioid antagonist such as **naltrexone** is administered. The antagonist effectively displaces the bound opioid from its receptor. Treatment of a conscious patient in this manner would cause a devastating acute withdrawal syndrome; hence, the patient is anesthetized. Physiologic adaptation to the presence of the antagonist then occurs. As the plasma concentration of the antagonist falls, the potential for serious withdrawal symptoms abates, and the patient is allowed to recover from the general anesthesia.

## SOCIAL TREATMENTS

As the case of J.B. illustrates, detoxifying a patient is not sufficient by itself to ensure long-term abstinence. The risk of relapse is high, and long-term management of addiction is needed to achieve continued sobriety. In general, the most effective management programs have involved social intervention or a combination of social and pharmacologic means. **12-Step programs** such as **Alcoholics Anonymous** (AA) emphasize specific positive steps that foster continued sobriety (Box 16-2). Foremost among these steps is the admission by the patient that the problem is addiction and that the only way to prevent a relapse is to maintain abstinence. AA and related programs provide lay support groups and mentoring; the presence of such help mitigates the sense of alienation and loneliness that is often felt by recovering drug and alcohol abusers. Other programs add to lay support and mentoring, by a trained professional who leads groups and helps counsel patients.

Another therapeutic stance toward alcohol abuse emphasizes moderation rather than abstinence. **Moderation management** focuses on establishing boundaries to drinking, and on taking steps to ensure that the patient stays within those boundaries. This is accomplished by helping the patient understand the context under which alcohol is being used and to avoid situations, such as drinking under excessive stress, that lead to problem drinking. Although there is much controversy over the effectiveness of moderation management in individuals who are clearly addicted to alcohol, this strategy has proved to be an effective way of managing alcohol use for some "problem drinkers," patients who often overindulge but who are not yet addicted.

## PHARMACOLOGIC TREATMENT

Although some have resisted using other drugs to treat chemical addiction, the finding that addiction is caused by fundamental changes in brain reward pathways has suggested that pharmacotherapy could have an important role in the management of addiction. To date, several pharmacologic strategies have been employed.

The first of these strategies is the chronic administration of an agent that causes extremely unpleasant side effects when the drug of abuse is used. For example, **disulfiram** inhibits aldehyde dehydrogenase, a critical enzyme in the alcohol metabolism pathway. In an individual who ingests ethanol while taking disulfiram, alcohol dehydrogenase oxidizes the ethanol to acetaldehyde, but disulfiram prevents aldehyde dehydrogenase from metabolizing the acetaldehyde. Therefore, this toxic metabolite accumulates in the blood. Acetaldehyde causes

### BOX 16-2. The 12-Step Program

The relative success of the Alcoholics Anonymous (AA) program seems to be attributable to the fact that an alcoholic in recovery (i.e., one who no longer drinks) has an exceptional faculty for "reaching out" and helping an uncontrolled drinker.

In its simplest form, the AA program operates when a recovered alcoholic passes along the story of his or her own problem drinking, describes the sobriety he or she has found in AA, and invites the newcomer to join the informal Fellowship.

The heart of the suggested program of personal recovery is contained in Twelve Steps describing the experience of the earliest members of the Society.

1. We admitted we were powerless over alcohol—that our lives had become unmanageable.
2. Came to believe that a Power greater than ourselves could restore us to sanity.
3. Made a decision to turn our will and our lives over to the care of God *as we understood Him.*
4. Made a searching and fearless moral inventory of ourselves.
5. Admitted to God, to ourselves, and to another human being the exact nature of our wrongs.
6. Were entirely ready to have God remove all these defects of character.
7. Humbly asked Him to remove our shortcomings.
8. Made a list of all persons we had harmed and became willing to make amends to them all.
9. Made direct amends to such people wherever possible, except when to do so would injure them or others.
10. Continued to take personal inventory and when we were wrong promptly admitted it.
11. Sought through prayer and meditation to improve our conscious contact with God *as we understood Him,* praying only for knowledge of His will for us and the power to carry that out.
12. Having had a spiritual awakening as the result of these steps, we tried to carry this message to alcoholics and to practice these principles in all our affairs.

Newcomers are not asked to accept or follow these Twelve Steps in their entirety if they feel unwilling or unable to do so. Instead, they are asked to keep an open mind, to attend meetings at which recovered alcoholics describe their personal experiences in achieving sobriety, and to read AA literature describing and interpreting the AA program.

AA members usually emphasize to newcomers that only problem drinkers themselves, individually, can determine whether or not they are in fact alcoholics.

At the same time, it is pointed out that all available medical testimony indicates that alcoholism is a progressive illness, that it cannot be cured in the ordinary sense of the term, but that it can be arrested through total abstinence from alcohol in any form. (Adapted from "The Twelve Steps of Alcoholics Anonymous.")

a number of unpleasant symptoms, including facial flushing, headache, nausea, vomiting, weakness, orthostatic hypotension, and respiratory difficulty. These symptoms can last from 30 minutes to several hours and are followed by exhaustion and fatigue. The unpleasant side effects of alcohol consumption in the presence of disulfiram are intended as a deterrent to further drinking. Unfortunately, the effectiveness of disulfiram is limited by failures in compliance.

Another strategy used to treat addiction is to block the effects of the drug of abuse. **Naltrexone** is an opioid antagonist that competitively blocks the binding of opioids to the opioid receptor. Thus, a patient who injects an opioid such as heroin while taking naltrexone will not experience the "high" that normally accompanies drug use. Studies have shown that naltrexone also acts as an opioid inhibitor in the brain reward pathway. Thus, the effects of a drug such as ethanol, which may share a final common reward pathway involving the opioid receptor, may also be inhibited by naltrexone. For this reason, naltrexone has been used to treat alcohol addiction, although a recent randomized, placebo-controlled clinical trial has called its clinical effectiveness into question. Naltrexone does not produce withdrawal effects by itself. Naltrexone is not generally administered when there are traces of exogenous opioids in the system, however, because naltrexone can antagonize any remaining drug, leading to the development or exacerbation of withdrawal symptoms. Although naltrexone can effectively prevent the "high" associated with opioid abuse, it does not alleviate cravings or withdrawal effects, and there is a relatively high likelihood of compliance lapses. Therefore, naltrexone has been effective only in individuals addicted to opioids or alcohol who have a high motivation to stay drug free.

A third pharmacologic approach has been the use of a slow-acting agonist. **Methadone,** as discussed above, is a slow-acting opioid agonist. Because it is taken orally, it does not produce the sharp increases in plasma levels required to elicit a "high" such as that accompanying the injection of heroin or other injected opioids. Methadone also has a very long half-life as compared to heroin or morphine. Thus, even once-daily administration of methadone produces plasma opioid levels that remain fairly constant over time and that, therefore, mitigate cravings (Fig.16-10). Moreover, methadone produces cross-tolerance to other opioids, so that a patient who injects heroin or another opioid while taking methadone will experience a reduced effect of the injected drug.

Recent pharmacologic approaches to treating opioid addiction have been further refined by using a partial opioid agonist such as **buprenorphine** (which has recently been approved for use in the United States). Because of its agonist effect, buprenorphine can alleviate cravings and withdrawal symptoms. On the other hand, because it is not a full agonist, buprenorphine carries a low risk of overdose, and it can antagonize the euphorigenic effects of a full agonist (such as heroin) should the patient "slip." In addition, the withdrawal effects of buprenorphine are mild compared to those of full opioid agonists. Thus, buprenorphine functions pharmacologically as would a combination of naltrexone and methadone.

Although various pharmacologic treatments are available for alcohol and opioid addiction, there is a paucity of current treatments for cocaine and amphetamine abuse. Several trials have attempted to use antidepressants, such as the tricyclic antidepressant **desipramine** or the selective serotonin reuptake inhibitor **fluoxetine.** Desipramine acts by blocking monoamine reuptake (especially norepinephrine reuptake), whereas fluoxetine inhibits serotonin reuptake. Both agents have been shown to reduce drug craving but, unfortunately, neither has been shown to prevent cocaine use.

## *Conclusion and Future Directions*

This chapter has discussed the major causes of chemical dependence and addiction. Drug dependence is caused by a homeostatic adaptation to the presence of the drug. In comparison, addiction is a harmful, out-of-control use of drugs associated with context-induced craving, especially under situations of stress, that can lead to relapse. This chapter has also considered the major drugs of abuse, including the misuse of prescription drugs and the abuse of nonprescription controlled substances. Although each drug has its own molecular and cellular mechanism of action, many abused drugs affect the brain reward pathway. Finally, this chapter has discussed the major treatments for dependence and addiction, including the pharmacologic prevention and treatment of withdrawal symptoms, the long-term social treatments of addiction, and some newer pharmacologic treatments that promote long-lasting sobriety.

As research into its mechanisms continues to support the notion that addiction is a long-term, chronic illness, akin to atherosclerosis or diabetes, new treatments for addiction will help in the long-term management of the disease. New directions in addiction research are exemplified by two very different attempts to treat cocaine abuse. First, drugs that interact with specific dopamine receptor subtypes have been explored, investigating the hypotheses that a D1-specific agonist could suppress drug cravings and a D2-specific antagonist could prevent the euphorigenic effects of cocaine. These hypotheses were generated based on experiments in mice, in which priming of the mice with a D1 agonist suppressed cocaine-seeking behavior.

Second, a novel vaccine approach has been investigated. Researchers injected a protein-conjugated analogue of cocaine into rats, and the rats produced antibodies directed against cocaine. Later, when the immunized rats were injected with cocaine, their brain cocaine levels were lower than those of unimmunized rats who were similarly injected. Based on these promising animal studies, researchers have initiated large-scale clinical trials of a cocaine vaccine, under the theory that cocaine will be less euphorigenic in vaccinated persons who are exposed to the drug. Trials of an analogous antinicotine vaccine are forthcoming. If successful, this approach could be extended to other drugs of abuse.

## *Acknowledgment*

The authors are indebted to Dr. David Potter for his generous help in the preparation of this chapter.

## *Suggested Readings*

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR). Washington DC: American Psychiatric Association, 2000.

Camí J, Farré M. Mechanisms of Disease: Drug Addiction. N Engl J Med 2003;349:975–986.

Hyman S. Addiction to cocaine and amphetamine. Neuron 1996; 16:901–904. (*Reviews how the psychostimulants interface with the brain reward pathway, and how neuronal adaptations to the drugs lead to addiction.*)

Hyman S. Why does the brain prefer opium to broccoli? Harvard Rev Psychiat 1994;43–46. (*Excellent introductory article explains the basics of addiction and dependence. It covers both the brain reward pathways and the adaptations made by cells in response to long-term drug abuse.*)

Leshner A. Molecular mechanism of cocaine addiction. N Engl J Med 1996;335128–129. (*Reviews research in cocaine addiction, and how this research could lead to novel treatments. Includes research into a possible cocaine vaccine.*)

Nestler E, Aghajanian G. Molecular and cellular basis of addiction. Science 1997;278:58–63. (*Describes addiction on a molecular and cellular basis, emphasizing changes in the cAMP pathway and the cytoskeleton.*)

Nestler E. Under siege, the brain on opiates. *Neuron.* 1996;16: 897–900. (*Explains how opioids cause addiction, focusing on psychologic dependence rather than the more well-studied physical dependence. Also explains cellular changes in the cAMP pathway in response to opioids.*)

O'Brien CP. A range of research-based pharmacotherapies for addiction. Science 1997;278:66–70. (*Reviews the principal long-term pharmacologic treatments for addiction, including the administration of antagonists, agonists, and partial agonists.*)

www.aa.org. (*This web site offers excellent information on Alcoholics Anonymous.*)

## Drug Summary Table Pharmacology of Chemical Dependence and Addiction

| Drug | Clinical Use | Side Effects/Toxicity | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **OPIOID ANTAGONISTS**<br>*Mechanism—Block opioid receptor; displace opioid from binding site* | | | | |
| **Naloxone** | Opioid overdose<br>Rapid detoxification | Hypertension, hypotension, tachycardia, ventricular arrhythmias, cardiac arrest, irritability, anxiety, opioid withdrawal, restlessness, seizures, nausea, vomiting, diarrhea, tremulousness, pulmonary edema | Interacts with opioid analgesics<br>Risk of pulmonary edema, especially in patients with CHF | Short half-life |
| **Naltrexone** | Prevent relapse of opioid abuse | Insomnia, headache, nausea, vomiting, dizziness, anxiety, somnolence, diarrhea, constipation, erectile dysfunction | Interacts with opioid analgesics<br>May precipitate acute withdrawal after chronic opioid use | Should give after a 7–10 day opioid-free period to prevent precipitation of withdrawal.<br>Long half-life compared to naloxone |
| **LONG-ACTING OPIOID AGONISTS**<br>*Mechanism—Bind and activate opioid receptors* | | | | |
| **Methadone**<br>**Levomethadyl acetate** | Prevent relapse of opioid abuse | CNS depression, chills, anxiety, euphoria, headache, insomnia, nervousness, hot flashes, constipation, diarrhea, xerostomia, nausea, vomiting, erectile dysfunction, miosis, physical and psychological dependence | Use in conjugation with opioid antagonist or partial agonist can cause withdrawal symptoms | Suppresses symptoms of withdrawal in opioid-dependent persons because of slow absorption and long half-life; can produce sufficient tolerance to block the subjective high of usual doses of parenterally administered opioids |
| **OPIOID PARTIAL AGONISTS**<br>*Mechanism—Bind to and partially antagonize opioid receptors* | | | | |
| **Buprenorphine** | Prevent relapse of opioid abuse | Sedation, hypotension, respiratory depression, dizziness, headache, vomiting, nausea, miosis, diaphoresis, bradycardia, constipation, depression, euphoria, nervousness | Use in conjugation with opioid antagonist can cause withdrawal symptoms; may produce an additive effect with other respiratory depressants | |

# III

# Principles of Cardiovascular Pharmacology

# 17

# Pharmacology of Cardiac Rhythm

April Wang Armstrong and David E. Clapham

**Introduction**
**Case**
**Electrical Physiology of the Heart**
  Pacemaker and Nonpacemaker Cells
  Cardiac Action Potentials
  Determination of Firing Rate
**Pathophysiology of Electrical Dysfunction**
  Defects in Impulse Formation (SA Node)
    *Altered Automaticity*
    *Triggered Activity*
  Defects in Impulse Conduction
    *Re-entry*
    *Conduction Block*
    *Accessory Tract Pathways*
**Pharmacologic Classes and Agents**
  General Mechanisms of Action of Antiarrhythmic Agents
  Classes of Antiarrhythmic Agents
    *Class I Antiarrhythmic Agents: Fast Na$^+$ Channel Blockers*
    *Class II Antiarrhythmic Agents: β-Adrenergic Blockers*
    *Class III Antiarrhythmic Agents: Inhibitors of Repolarization*
    *Class IV Antiarrhythmic Agents: Ca$^{2+}$ Channel Blockers*
    *Other Antiarrhythmic Agents: Adenosine and Potassium*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

The human heart is both a mechanical and an electrical organ. To perfuse the body adequately with blood, the mechanical and electrical components of the heart must work in precise concert with each other. The mechanical component pumps the blood; the electrical component controls the rhythm of the pump. When the mechanical component fails despite a normal rhythm, congestive heart failure can result (see Chapter 23, Integrative Cardiovascular Pharmacology). When the electrical component goes awry (called an arrhythmia), cardiac myocytes fail to contract in synchrony, and effective pumping is compromised. Changes in the membrane potential of cardiac cells directly affect cardiac rhythm, and most antiarrhythmic drugs act by modulating the activity of ion channels in the plasma membrane. This chapter discusses the ionic basis of electrical rhythm formation and conduction in the heart, the pathophysiology of electrical dysfunction, and the pharmacologic agents used to restore a normal cardiac rhythm.

## Case

One winter morning, Dr. Johnson, a 56-year-old professor, is lecturing on the development of cardiomyopathies to the second-year medical school class. He feels his heart beating irregularly and becomes nauseated. The students in the front row notice that he is sweating profusely, and is pale and short of breath. Dr. Johnson immediately puts down his laser pointer and apologizes to the students, saying, "We might have to cut this lecture short." He sits down suddenly, grabs his chest, and croaks, "I feel like an elephant is sitting on my chest."

One quick-thinking student runs out and dials 911. The EMTs quickly arrive on the scene and assess Dr. Johnson. He has a blood pressure of 90/60 and a heart rate of 120 beats per minute. The EKG shows that Dr. Johnson is in ventricular tachycardia. Dr. Johnson receives a continuous IV infusion of lidocaine and is given sublingual nitroglycerin. His heart rate decreases, his blood pressure increases, and he reports feeling much better.

After Dr. Johnson is transported to the nearest hospital, a car accident involving eight people requires the immediate attention of the ER staff. While most of the ER staff is caring for the car crash victims, Dr. Johnson's IV line kinks, and the lidocaine infusion stops abruptly. Twenty minutes later, he experiences palpitations and calls the nurse. By the time the nurse arrives, he has become unconscious and pulseless. CPR is begun, and an EKG shows that the professor is in ventricular fibrillation. The ER staff quickly cardioverts Dr. Johnson. After Dr. Johnson's heart condition is stabilized, he is referred to a cardiologist for further evaluation.

## QUESTIONS

- **1.** Why did the paramedics administer lidocaine to Dr. Johnson?
- **2.** In addition to its use as an antiarrhythmic agent, in what other clinical situations can lidocaine be used therapeutically?
- **3.** Why was sublingual nitroglycerin administered in combination with lidocaine at the onset of Dr. Johnson's symptoms?
- **4.** Why did Dr. Johnson experience palpitations 20 minutes after the lidocaine infusion was inadvertently discontinued?

## ELECTRICAL PHYSIOLOGY OF THE HEART

Electrical cycling of the heart, leading to rhythmic cardiac contraction, is a manifestation of the heart's exquisite control of cell depolarization and impulse conduction. Once initiated, a cardiac action potential is a spontaneous event that relies on the characteristic responses of ion channels to changes in membrane voltage. At the completion of a cycle, the spontaneous depolarization of pacemaker cells ensures that the process repeats over and over, without interruption.

### PACEMAKER AND NONPACEMAKER CELLS

The heart contains two types of cardiac myocytes—those that can spontaneously initiate action potentials and those that cannot. Cells possessing the ability to initiate spontaneous action potentials are termed **pacemaker cells.** All pacemaker cells possess **automaticity,** the ability to depolarize above a threshold voltage in a rhythmic fashion. Automaticity results in the generation of spontaneous action potentials. Pacemaker cells are found in the sinoatrial node (SA node), the atrioventricular node (AV node), and the ventricular conducting system (bundle of His, bundle branches, and Purkinje fibers). Together, the pacemaker cells constitute the specialized conducting system that governs the electrical activity of the heart. The second type of cardiac cells, the **nonpacemaker cells,** include the atrial and ventricular myocytes. The nonpacemaker cells contract in response to depolarization and are responsible for the majority of cardiac contraction. In *pathologic* conditions, these nonpacemaker cells can acquire automaticity and, thereby, also act as pacemaker cells.

### CARDIAC ACTION POTENTIALS

Ions are not distributed equally across cell membranes. Transporters (pumps) drive $K^+$ into cells while pumping $Na^+$ and $Ca^{2+}$ out, giving rise to electrical and chemical gradients across the membrane. These gradients ultimately determine the membrane potential of a cardiac cell. The **Nernst equilibrium potential** for each ion ($E_{Na} = +70$ mV, $E_K = -94$ mV, and $E_{Ca} = +150$ mV) depends on the relative concentrations of ions inside and outside the cell. The difference between an ion's Nernst potential and the cell's membrane potential determines the driving force for ions into or out of the cell. Refer to Chapter 5, Principles of Cellular Excitability and Electrochemical Transmission, for a detailed discussion of the Nernst equilibrium potential.

When an ion-specific channel opens, the membrane potential approaches the equilibrium potential for that ion. For example, opening a $K^+$-selective channel drives the membrane potential toward $E_K$ (−94 mV). The final membrane potential depends on the number of channels, their conductances (i.e., the ability of each channel to pass ions), and the duration for which each channel remains open. *The resting membrane is relatively permeable to $K^+$ ions but not to $Na^+$ or $Ca^{2+}$ ions;* hence, the **resting membrane potential** is close to the equilibrium potential for $K^+$.

Changing the membrane potential requires the movement of relatively few ions across the membrane. Because of this, the ionic concentration gradients across the membrane remain relatively stable, and the Nernst potential for each ion is constant, despite the opening and closing of ion channels.

Cardiac action potentials are strikingly longer than those of nerve or muscle, lasting for almost half a second. Prolonged cardiac action potentials provide the sustained depolarization and contraction needed to empty the heart's chambers. Sinoatrial (SA) nodal cells pace the heart at normal resting heart rates between 60 and 100 beats/min, while ventricular-muscle cells orchestrate the contraction that ejects blood from the heart (Fig. 17-1).

SA nodal cells fire spontaneously in a cycle defined by three phases, referred to as phase 4, phase 0, and phase 3 (Fig. 17-2 and Table 17-1). **Phase 4** consists of a slow spontaneous depolarization (accounting for SA node automaticity) that is caused by an inward ($I_f$) current (carried mainly by $Na^+$ ions). The channels that carry the $I_f$ current are activated during the repolarization phase of the previous action potential. **Phase 0** consists of highly selective voltage-gated $Ca^{2+}$ channels that, upon opening, drive the membrane potential towards $E_{Ca}$. In **phase 3,** the $Ca^{2+}$ channels slowly close, and $K^+$-selective channels open, resulting in membrane repolarization. Once the membrane potential repolarizes to approximately −60 mV, the opening of $I_f$ channels is triggered, and the cycle begins again.

Unlike SA nodal cells, ventricular myocytes do not depolarize spontaneously under physiologic conditions. As a result, the membrane potential of the resting ventricular myocyte remains near $E_K$ until the cell is stimulated by a wave of depolarization that is initiated by nearby pacemaker cells. The five phases of the ventricular myocyte action potential result from an intricately woven cascade of channel openings and closings; the phases are numbered from 0 to 4 (Fig. 17-3 and Table 17-1).

In **phase 0,** an action potential upstroke of rapid depolarization is caused by a transient increase in inward $Na^+$ current through the voltage-gated $Na^+$ channel. (Note that currents in phase 0 of the SA nodal and ventricular myocyte action potentials are carried by different ions—$Ca^{2+}$ and $Na^+$, respectively.) The opening of $Na^+$ channels ($I_{Na}$) leads to a rapid influx of $Na^+$, which accounts for the depolarization and drives the membrane potential toward $E_{Na}$ (+70 mV). Although large, the increase in $Na^+$ conductance during phase 0 lasts for only 1–2 milliseconds, because the $Na^+$ channels inactivate as a function of time and voltage. Inactivation of the fast $Na^+$ channels causes a dramatic decrease in the inward $Na^+$ cur-

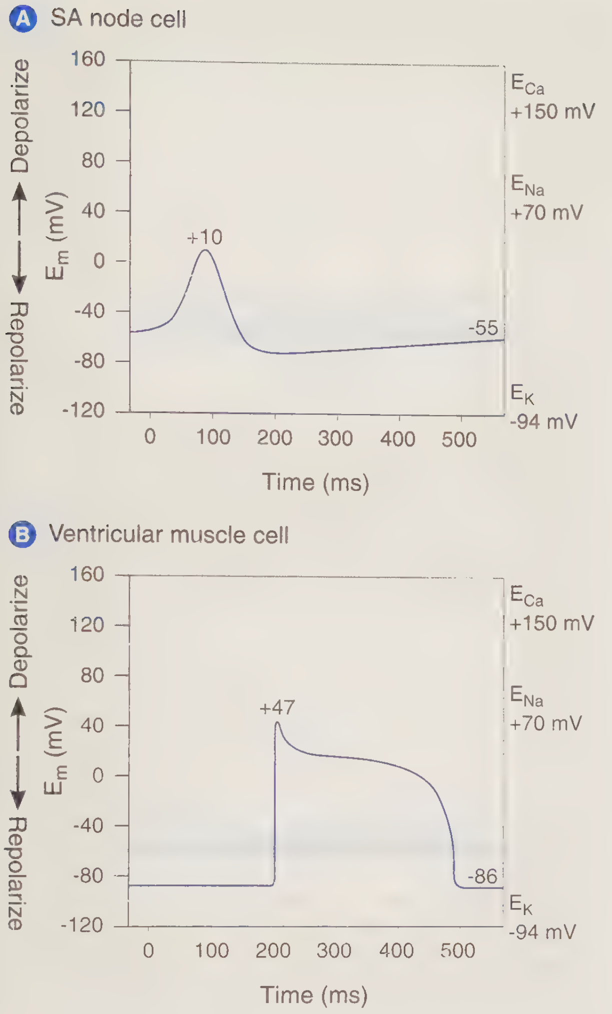


**Figure 17-1. SA Node and Ventricular Muscle Cell Action Potentials.** The resting membrane potential of a sinoatrial (SA) node cell is approximately –55 mV, while that of a ventricular muscle cell is –86 mV. The shaded areas represent the approximate depolarization required to trigger an action potential in each cell type. Together, the cardiac action potentials last for approximately half a second. SA node cells depolarize to a peak of +10 mV, and ventricular muscle cells depolarize to a peak of +47 mV. Note that the ventricular action potential has a much longer plateau phase. This long plateau ensures that ventricular myocytes have adequate time to contract before the onset of the next action potential. The Nernst equilibrium potentials of the major ions ($E_{Ca}$, $E_{Na}$, $E_K$) are shown as dashed horizontal lines. $E_m$, membrane potential.

rent. The time it takes for $Na^+$ channels to recover from their voltage-dependent and time-dependent inactivation determines the *refractory period* of the myocyte. The refractory period is the time during which another action potential cannot fire. This serves as a protective mechanism to ensure that the heart has sufficient time to eject blood from its chambers. The refractory period lasts from the initiation of the action potential upstroke until the repolarization phase. $I_{Na}$ is the

| Phases of SA Node Action Potential | Major Currents |
|---|---|
| Phase 4 | $I_f$ = Pacemaker current, mainly inward $Na^+$ current. $I_{K_1}$ = Inward rectifier, outward $K^+$ current |
| Phase 0 | $I_{Ca}$ = Inward $Ca^{2+}$ current |
| Phase 3 | $I_K$ = Delayed rectifier, outward $K^+$ current |

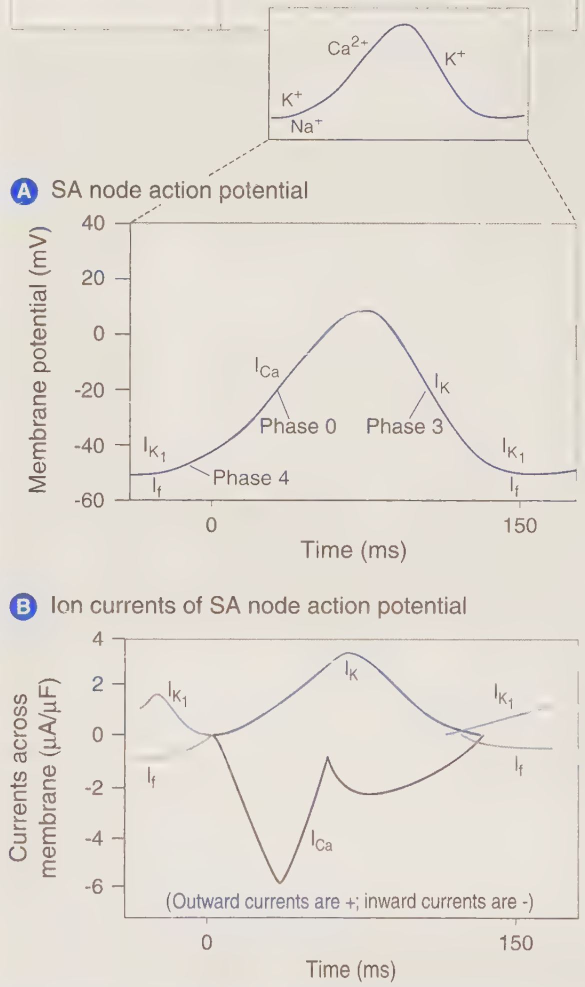


**Figure 17-2. SA Node Action Potential and Ion Currents. A.** SA nodal cells are depolarized slowly by the pacemaker current ($I_f$) (phase 4), which consists of an inward flow of sodium (mostly) and calcium ions. Depolarization to the threshold potential opens highly selective voltage-gated calcium channels, which drive the membrane potential towards $E_{Ca}$ (phase 0). As the calcium channels close and potassium channels open (phase 3), the membrane potential repolarizes. **B.** The flux of each ion species correlates roughly with each phase of the action potential. Positive currents indicate an outward flow of ions (blue), while negative currents are inward (gray).

**TABLE 17-1** Major Characteristics of Action Potential Phases for SA Nodal Cells and Ventricular Myocytes

| Segment | Characteristics | Major underlying current |
|---|---|---|
| **SA Nodal Cells** | | |
| Phase 4 | Slow depolarization | Inward $I_f$ current (carried mainly by $Na^+$ ions) |
| Phase 0 | Action potential upstroke | Inward $Ca^{2+}$ current through voltage-sensitive $Ca^{2+}$ channels |
| Phase 3 | Repolarization | Outward $K^+$ current through $K^+$ channels |
| **Ventricular Myocytes** | | |
| **Segment** | **Characteristics** | **Major underlying current** |
| Phase 4 | Resting membrane potential | Inward and outward currents are equal |
| Phase 0 | Rapid depolarization | Inward $Na^+$ current through $Na^+$ channels |
| Phase 1 | Early phase of repolarization | Decrease in inward $Na^+$ current and efflux of $K^+$ ions through $K^+$ channels ($I_{to}$) |
| Phase 2 | Plateau | Balance between inward $Ca^{2+}$ current through $Ca^{2+}$ channels and outward $K^+$ current through $K^+$ channels ($I_K$, $I_{K1}$) |
| Phase 3 | Late phase of rapid repolarization | Decrease in inward $Ca^{2+}$ current and large increase in outward $K^+$ current |

major determinant of the velocity of impulse conduction throughout the ventricle.

The threshold-dependent activation of $I_{Na}$ quickly depolarizes the membrane. The upstroke terminates before reaching $E_{Na}$, however, and is followed by an early phase of rapid repolarization to about +20 mV. This **phase 1** repolarization is a consequence of two events: 1) the rapid voltage-dependent inactivation of $I_{Na}$; and 2) the activation of transient $K^+$ currents ($I_{to}$; transient outward).

**Phase 2,** the plateau of the action potential, is maintained by a finely tuned balance between an inward $Ca^{2+}$ current through two types of $Ca^{2+}$ channels ($I_{Ca.T}$, $I_{Ca.L}$) and an outward $K^+$ current through several types of $K^+$ channels ($I_K$, $I_{K1}$, $I_{to}$). Remarkably, only a few hundred channels per cell are used to maintain this fine balance. The plateau phase of the ventricular action potential is unique to cardiac cell electrophysiology. Because only a small number of channels are open, the total membrane conductance is low. The high membrane resistance during the plateau phase insulates the cardiac cells electrically, allowing rapid propagation of the action potential with little current dissipation.

During the plateau phase, two distinct $Ca^{2+}$ currents—the transient $Ca^{2+}$ current, $I_{Ca.T}$, and the long-lasting $Ca^{2+}$ current, $I_{Ca.L}$—mediate the influx of $Ca^{2+}$ needed to initiate contraction. T-type $Ca^{2+}$ channels inactivate with time and are insensitive to block by dihydropyridines such as nifedipine and nitrendipine. Current through the L-type $Ca^{2+}$ channels ($I_{Ca.L}$) provides the dominant $Ca^{2+}$ current in virtually all cardiac cells. $I_{Ca.L}$ is activated at −30 mV and inactivates slowly (hundred of milliseconds). It is sensitive to block by dihydropyridines (nifedipine), benzothiazepines (diltiazem), and phenylalkylamines (verapamil), as discussed below. L-type $Ca^{2+}$ channels carry inward current throughout the plateau phase, and because $Ca^{2+}$ stimulates the contraction of cardiac myocytes, are crucial for coupling membrane excitability to myocardial contraction.

Outward currents through the $K^+$ channels that are activated during the plateau phase oppose the inward $Ca^{2+}$ currents. As the time-dependent inward $Ca^{2+}$ currents inactivate, the outward $K^+$ currents (mostly $I_K$) rapidly drive the membrane potential toward $E_K$, thus repolarizing the cell in **phase 3.** However, these channels are unable to drive the membrane potential all the way to $E_K$ because they deactivate at −40 mV. In **phase 4,** the resting membrane potential is reestablished by the activation of time-independent $K^+$ currents ($I_{K1}$), which drives the membrane potential close to the $K^+$ equilibrium potential.

In clinical practice, the overall electrical activity of the heart is measured, rather than the ionic changes that occur at a single-cell level. This overall activity is reported in the electrocardiogram, or EKG (Box 17-1 and Fig. 17-4).

## DETERMINATION OF FIRING RATE

The specialized conduction system of the heart consists of the SA node, AV node, bundle of His, and Purkinje system. These different populations of cells have different intrinsic rates of firing. Three factors determine the firing rate. First, as the rate of spontaneous depolarization in phase 4 increases, the rate of firing increases. Second, as the threshold potential (the minimum potential necessary to trigger an action potential) becomes more negative, the threshold potential is more quickly reached at the end of phase 4, and the rate of firing increases. Third, as the maximum negative diastolic potential (the resting membrane potential) becomes more positive, less time is needed to repolarize the membrane fully at the end of phase 3, and the rate of firing increases.

Because the various populations of pacemaker cells possess different intrinsic rates of firing, the pacemaker population with the fastest firing rate sets the heart rate. The SA node possesses the fastest intrinsic firing rate—60–100 times per minute—and is the **native pacemaker** of the heart. The cells of the atrioventricular (AV) node and bundle of His fire intrinsically between 50 and 60 times per minute, and the cells of the Purkinje system have the slowest intrinsic firing rate—30–40 times per minute. The cells of the AV node, bundle of His, and Purkinje system are termed **latent pace-**

| Phases of Ventricular Action Potential | Major Currents |
|---|---|
| Phase 4 | $I_{K_1}$ = Inward rectifier, outward $K^+$ current<br>$I_{Na/Ca}$ = Inward $Na^+$ and $Ca^{2+}$ current |
| Phase 0 | $I_{Na}$ = Fast inward $Na^+$ current |
| Phase 1 | $I_{to}$ = Transient outward $K^+$ current |
| Phase 2 | $I_{Ca}$ = Inward $Ca^{2+}$ current<br>$I_K$ = Delayed rectifier, outward $K^+$ current<br>$I_{K_1}$ = Inward rectifier, outward $K^+$ current<br>$I_{to}$ = Transient outward $K^+$ current |
| Phase 3 | $I_K$ = Delayed rectifier, outward $K^+$ current |

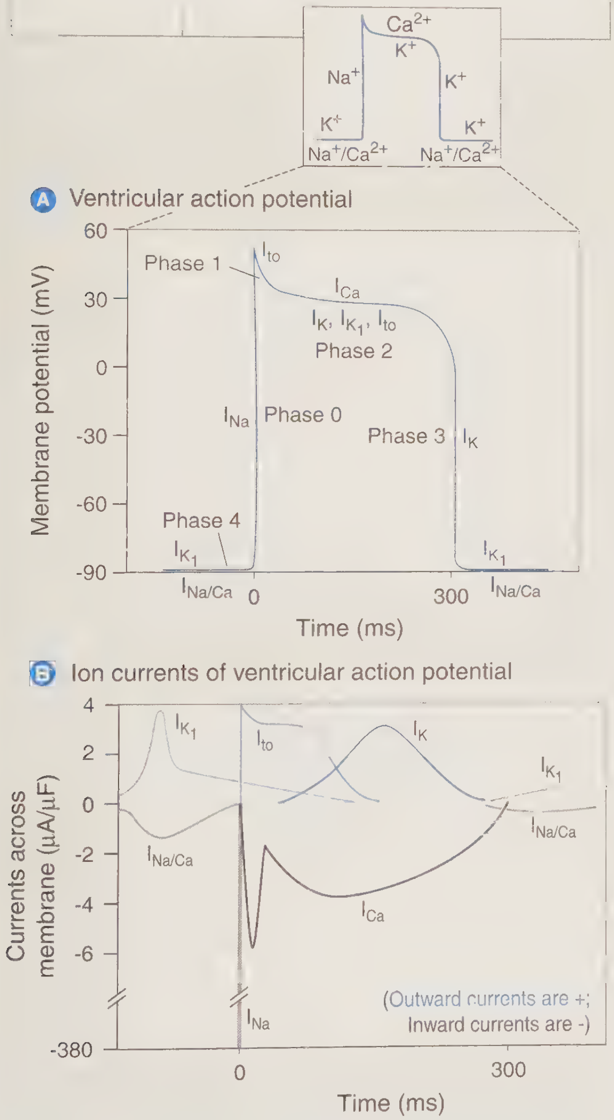


**Figure 17-3. Ventricular Action Potential and Ion Currents.** **A.** At the resting membrane potential (phase 4), the inward and outward currents are equal and the membrane potential approaches the K⁺ equilibrium potential ($E_K$). During the action potential upstroke (phase 0), there is a large transient increase in Na⁺ conductance. This is followed by a brief period of initial repolarization (phase 1), which is mediated by a transient outward K⁺ current. The plateau of the action potential (phase 2) results from the opposition of an inward $Ca^{2+}$ current and an outward $K^+$ current. The membrane repolarizes (phase 3) when the inward $Ca^{2+}$ current decreases and the outward $K^+$ current predominates. **B.** The ion fluxes that give rise to the ventricular action potential consist of a complex pattern of changing ion permeabilities that are separated in time. Note especially that the $Na^+$ current in phase 0 is very large but extremely brief.

### BOX 17-1. The Electrocardiogram

The electrocardiogram (EKG) is used to infer changes in cardiac impulses by recording electrical potentials at various locations on the surface of the body. An EKG recording reflects changes in the excitation of the myocardium. A basic understanding of the EKG is useful for later discussions of the clinical applications of the various antiarrhythmic agents.

A normal electrocardiogram contains three electrical waveforms: the P wave, the QRS complex, and the T wave (Fig. 17-4). The **P wave** represents *atrial depolarization;* the **QRS complex** represents *ventricular depolarization;* and the **T wave** represents *ventricular repolarization.* The EKG does not show atrial repolarization, because the atrial repolarization is "drowned out" by the QRS complex. The EKG also contains two intervals and one segment: the PR interval, the QT interval, and the ST segment. The **PR interval** spans from the beginning of the P wave (initial depolarization of the atria) to the beginning of the Q wave (initial depolarization of the ventricles). Hence, the length of the PR interval varies with conduction velocity through the AV node. For example, if a patient has an electrical block in the AV node, then the conduction velocity through the AV node decreases and the PR interval increases. The **QT interval** spans from the beginning of the Q wave to the end of the T wave, representing the entire sequence of ventricular depolarization and repolarization. Last, the **ST segment** extends from the end of the S wave to the beginning of the T wave; this segment, which represents the period during which the ventricles are depolarized, corresponds to the plateau phase of the ventricular action potential.

**makers,** because their intrinsic rhythm is overridden by the faster SA node automaticity. In a mechanism termed **overdrive suppression,** the SA node suppresses the intrinsic rhythm of the other pacemaker populations and entrains them to fire at the SA nodal firing rate.

## PATHOPHYSIOLOGY OF ELECTRICAL DYSFUNCTION

Causes of electrical dysfunction in the heart can be divided into defects in impulse formation and defects in impulse conduction. In the former case, SA node automaticity is interrupted or altered, leading to missed beats or ectopic beats,

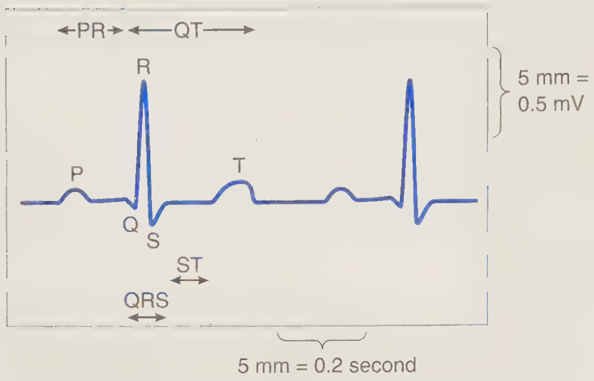


**Figure 17-4. Electrocardiogram.** The electrocardiogram (ECG or EKG) measures the body surface potentials induced by cardiac electrical activity. The **P wave** reflects *atrial depolarization,* the **QRS complex** represents *ventricular depolarization,* and the **T wave** indicates *ventricular repolarization.* The **PR interval** spans from the beginning of the P wave (initial depolarization of the atria) to the beginning of the Q wave (initial depolarization of the ventricles). The **QT interval** spans from the beginning of the Q wave to the end of the T wave, representing the entire interval of ventricular depolarization and repolarization. The **ST segment** extends from the end of the S wave to the beginning of the T wave, representing the period during which the ventricles are depolarized (i.e., the plateau phase of the action potential).

respectively. In the latter case, when impulse conduction is affected (for example, in the case of re-entrant rhythms), sustained arrhythmias can result.

## DEFECTS IN IMPULSE FORMATION (SA NODE)

As the native pacemaker of the heart, the SA node has a pivotal role in normal impulse formation. Electrical events that alter SA nodal function or disturb overdrive suppression can lead to impaired impulse formation. Two mechanisms commonly associated with defective impulse formation are altered automaticity and triggered activity.

### Altered Automaticity

Some mechanisms that alter automaticity of the SA node are physiologic. In particular, the autonomic nervous system often modulates automaticity of the SA node as part of a normal response. In sympathetic stimulation during exercise, an increased concentration of catecholamines leads to greater $\beta_1$-adrenergic receptor activation. Activation of $\beta_1$ receptors causes the opening of a greater number of pacemaker channels ($I_f$ channels); a larger pacemaker current is then conducted through these channels; and faster phase 4 depolarization results. Sympathetic stimulation also causes the opening of a greater number of $Ca^{2+}$ channels and, thereby, lowers threshold potential. Both of these mechanisms increase heart rate. The parasympathetic vagus nerve affects the SA node by a number of mechanisms that oppose the sympathetic regulation of heart rate. Vagal stimulation reduces the pacemaker current by decreasing the probability of pacemaker channel opening; it also increases threshold potential by reducing the probability of $Ca^{2+}$ channel opening; last, it makes the maximum diastolic potential (equivalent to the resting membrane potential in these spontaneously firing cells) more negative by increasing the probability of $K^+$ channel opening. The SA node, atria, and AV node are more sensitive than the ventricular conducting system to the effects of vagal stimulation.

In pathologic conditions, automaticity can be altered when latent pacemaker cells take over the SA node's role as the pacemaker of the heart. When the SA nodal firing rate becomes pathologically *slow* or when conduction of the SA impulse is impaired, an **escape beat** may occur as a latent pacemaker initiates an impulse. A series of escape beats known as an **escape rhythm** may result from prolonged SA nodal dysfunction. On the other hand, an **ectopic beat** occurs when latent pacemaker cells develop an intrinsic rate of firing that is *faster* than the SA nodal rate, in some cases despite the presence of a normally functioning SA node. A series of ectopic beats termed an **ectopic rhythm** can result from ischemia, electrolyte abnormalities, or heightened sympathetic tone.

Direct tissue damage (such as can occur after a myocardial infarction) also results in altered automaticity. Tissue injury can result in structural disruptions in the cell membrane. Disrupted membranes are unable to maintain ion gradients, which are critical for maintaining appropriate membrane potentials. If the resting membrane potential becomes sufficiently positive (more positive than –60 mV), nonpacemaker cells may begin to depolarize spontaneously. Another mechanism by which tissue damage leads to altered automaticity is through the loss of gap junction connectivity. Direct electrical connectivity is important for the effective delivery of overdrive suppression from the SA node to the rest of the cardiac myocytes. When connectivity is disrupted due to tissue injury, overdrive suppression is not efficiently relayed, and the unsuppressed cells initiate their own rhythm. This abnormal rhythm can lead to cardiac arrhythmia.

### Triggered Activity

**Afterdepolarizations** occur when a *normal* action potential triggers extra *abnormal* depolarizations. That is, the first action potential triggers additional oscillations of membrane potential, which may lead to arrhythmia. There are two types of afterdepolarizations—early afterdepolarizations and delayed afterdepolarizations.

If the afterdepolarization occurs *during* the inciting action potential, it is termed an **early afterdepolarization** (Fig. 17-5). *Situations that prolong the action potential (e.g., drugs that prolong the QT interval, such as procainamide and ibutilide) tend to trigger early afterdepolarizations.* Specifically, an early afterdepolarization can occur during the plateau stage (phase 2) or the rapid repolarization stage (phase 3). During the plateau phase, because most of the $Na^+$ channels are inactivated, an inward $Ca^{2+}$ current is responsible for the early afterdepolarization. On the other hand, during the rapid repolarization phase, partially recovered $Na^+$ channels can conduct an inward $Na^+$ current that contributes to the early afterdepolarization. If an early afterdepolarization is sustained, it can lead to a type of ventricular arrhythmia termed ***torsades de pointes.*** *Torsades de pointes,* French for "twisting of the

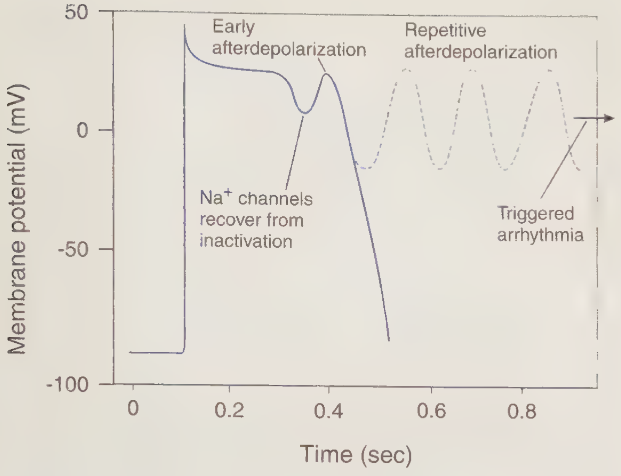


**Figure 17-5. Early Afterdepolarization.** Early afterdepolarizations generally occur during the repolarizing phase of the action potential, although they can also occur during the plateau phase. Repetitive afterdepolarizations can trigger an arrhythmia.

points," is characterized by QRS complexes of varying amplitudes as they "twist" along the baseline.

In contrast to early afterdepolarizations, **delayed afterdepolarizations** occur shortly *after the completion of repolarization* (Fig. 17-6). The mechanism of delayed afterdepolarizations is not well understood; it has been proposed that high intracellular $Ca^{2+}$ concentration leads to an inward $Na^+$ current, which, in turn, triggers the delayed afterdepolarization.

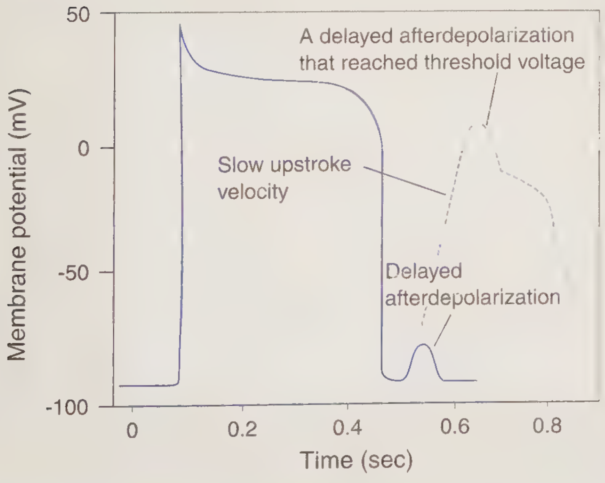


**Figure 17-6. Delayed Afterdepolarization.** Delayed afterdepolarizations occur shortly after repolarization. Although the mechanism has not been firmly elucidated, it appears that intracellular $Ca^{2+}$ accumulation activates the $Na^+/Ca^{2+}$ exchanger, and the resulting electrogenic influx of 3 $Na^+$ for each extruded $Ca^{2+}$ depolarizes the cell.

## DEFECTS IN IMPULSE CONDUCTION

The second type of electrical disturbance of the heart involves defects in impulse conduction. Normal cardiac function requires unobstructed and timely propagation of an electrical impulse through the cardiac myocytes. In pathologic conditions, altered impulse conduction can result from one or a combination of three mechanisms: re-entry, conduction block, and accessory tract pathways.

### Re-entry

Normal cardiac conduction is initiated at the SA node and propagated to the AV node, bundle of His, Purkinje fibers, and myocardium in an orderly fashion. The cellular refractory period ensures that stimulated regions of the myocardium depolarize only once during propagation of an impulse. Figure 17-7A depicts normal impulse conduction, in which an impulse arriving at point a travels synchronously down two parallel pathways, 1 and 2.

**Re-entry** of an electrical impulse occurs when a self-sustaining electrical circuit stimulates an area of the myocardium repeatedly and rapidly. Two conditions must be present for a re-entrant electrical circuit to occur: (1) *unidirectional block* (anterograde conduction is prohibited, but retrograde conduction is permitted); and (2) *slowed retrograde conduction velocity.* Figure 17-7B shows a reentrant electrical circuit. As the impulse arrives at point a, it can travel only down pathway 1 (the left branch), because pathway 2 (the right branch) is blocked *unidirectionally* in the anterograde direction. The impulse conducts through pathway 1 and travels to point b. At this junction, the impulse travels in a *retrograde* fashion up pathway 2 toward point a. The conduction time from point b to point a is slowed because of cell damage or the presence of cells that are still in the refractory state. By the time the impulse reaches point a, the cells in pathway 1 have had adequate time to repolarize and are, therefore, stimulated to continue conducting the action potential toward point b. In this manner, tachyarrhythmias result from the combination of unidirectional block and decreased conduction velocity in the abnormal pathway.

### Conduction Block

Conduction block occurs when an impulse fails to propagate because of the presence of an area of unexcitable cardiac tissue. This area of unexcitable tissue could consist of normal tissue that is still refractory, or it could represent tissue that has been damaged by trauma, ischemia, or scarring. In both cases, the myocardium is unable to conduct an impulse. Because conduction block removes overdrive suppression by the SA node, the cardiac myocytes are free to beat at their intrinsically slower frequency. For this reason, conduction block can be manifested clinically as bradycardia.

### Accessory Tract Pathways

During the normal cardiac cycle, the SA node initiates an impulse that travels quickly through the atrial myocardium and arrives at the AV node. Impulse conduction then slows through the AV node, allowing sufficient time for filling of the ventricles with blood before ventricular contraction is

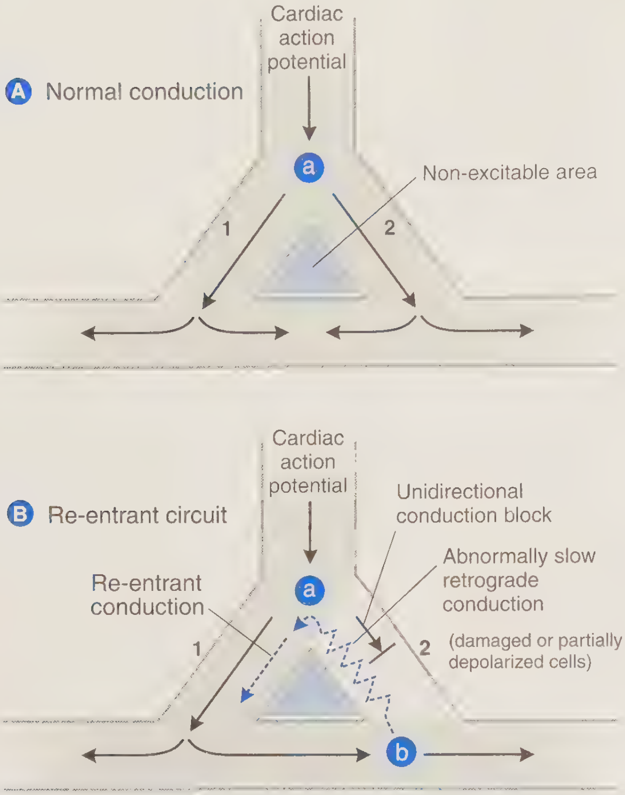


**Figure 17-7. Normal and Re-entrant Electrical Pathways.** **A.** In normal impulse conduction, an impulse traveling down a pathway arrives at point a, where its is able to travel down two alternate pathways, 1 and 2. In the absence of re-entry, the impulses continue on and depolarize different areas of the ventricle. **B.** A re-entrant circuit can develop if one of the branch pathways is pathologically disrupted. When the impulse arrives at point a, it can travel only down pathway 1 because pathway 2 is blocked *unidirectionally* (i.e., the effective refractory period of the cells in pathway 2 is prolonged to such an extent that anterograde conduction is prohibited). The impulse conducts through pathway 1 and proceeds to point b. At this point, the cells in pathway 2 are no longer refractory, and the impulse conducts in a retrograde fashion up pathway 2 toward point a. When the retrograde impulse arrives at point a, it can initiate re-entry. Re-entry can result in a sustained pattern of rapid depolarizations that trigger tachyarrhythmias. This mechanism can occur over small or large regions of the heart.

initiated. After the impulse travels through the AV node, it then propagates throughout the ventricles to trigger ventricular contraction.

Some individuals possess accessory electrical pathways that bypass the AV node. One common accessory pathway is the **bundle of Kent,** a band of myocardium that conducts impulses directly from the atria to the ventricles, bypassing the AV node (Fig. 17-8). In these individuals, an impulse originating in the SA node is conducted through the bundle of Kent to the ventricles more rapidly than the same impulse would be conducted through the AV node. Because the bundle of Kent is an *accessory* pathway, the ventricular tissue receives impulses from both the normal conduction pathway and the accessory pathway. As a result, electrocardiograms from these individuals usually exhibit a wider-than-normal QRS complex and an earlier-than-normal ventricular upstroke. More importantly, because the two conduction tracts have different conduction velocities, the presence of an accessory tract can set up the conditions for a re-entrant loop and, thereby, predispose the individual to tachyarrhythmias.

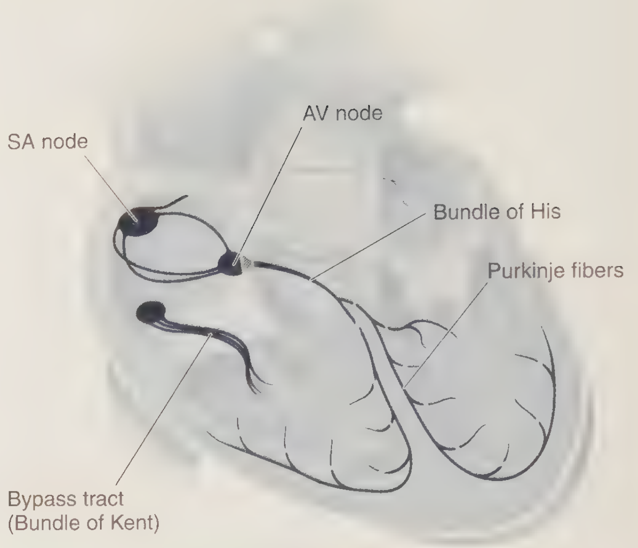


**Figure 17-8. Bundle of Kent.** The bundle of Kent is an accessory electrical pathway that conducts impulses directly from the atria to the ventricles, bypassing the AV node. Impulse conduction through this accessory tract is more rapid than conduction through the AV node, setting up the conditions for reentrant tachyarrhythmias.

## PHARMACOLOGIC CLASSES AND AGENTS

Ion currents across the plasma membrane induce changes in the membrane potential of cells. Changes in the membrane potential of cardiac pacemaker cells underlie the timely contraction of cardiac myocytes. Defects in impulse formation and altered impulse conduction can lead to disturbances in cardiac rhythm. Antiarrhythmic agents are used to restore normal cardiac rhythm by targeting proarrhythmic regions of the heart. *Most antiarrhythmic agents interact preferentially with particular states of ion channels and, thereby, block the current responsible for eliciting the arrhythmia.*

## GENERAL MECHANISMS OF ACTION OF ANTIARRHYTHMIC AGENTS

Although there are many different antiarrhythmic agents, there are surprisingly few mechanisms of antiarrhythmic action. In general, drugs that affect cardiac rhythm act by altering: (1) the maximum diastolic potential in pacemaker cells (and/or the resting membrane potential in ventricular cells); (2) phase 4 depolarization; (3) the threshold potential; or (4) the action potential duration. The specific effect of a particular channel blocker follows directly from the role of the current carried by that channel in the cardiac action potential. For example, $Na^+$ and $Ca^{2+}$ channel blockers typically alter the threshold potential, while $K^+$ channel blockers tend to prolong action potential duration. These drugs can access the ion channel by either traversing the pore of the channel or entering the lipid bilayer within which the channel is embedded.

**State-dependent ion channel block** is an important concept in antiarrhythmic drug action. Ion channels are capable of switching among various conformational states, and changes in the permeability of the membrane to a particular ion are mediated by conformational changes in the channels that pass that ion. Antiarrhythmic drugs often have different affinities for different conformational states of the ion channel; that is, these drugs bind to one conformation of the channel with higher affinity than they do to other conformations of the channel. This type of binding is referred to as "state-dependent."

$Na^+$ channel blockers serve as an excellent example to illustrate the concept of state-dependent ion channel block. The $Na^+$ channel undergoes three major state changes (open-closed-inactivated) throughout the course of an action potential. During the upstroke, the channel is in the open conformation. The channel becomes inactivated during the plateau phase, and it changes again to the resting (closed) conformation as the membrane is repolarized to its resting potential. Most $Na^+$ channel blockers bind preferentially to the open and inactivated states of the $Na^+$ channel, not to the resting (closed) state of the channel. In this way, the drugs tend to block the channels during the action potential (cardiac systole) and to dissociate from the channels during diastole.

The unblocking rate (i.e., dissociation rate) of the various $Na^+$ channel blockers is an important determinant of the steady-state block of $Na^+$ channels. For example, when heart rate increases, the time available for unblocking (dissociation of the drug from its binding site on the channel) decreases and, therefore, the degree of steady-state $Na^+$ channel block increases. The action of $Na^+$ channel blockers on ischemic tissue illustrates the therapeutic utility of state-dependent block. It has been observed that $Na^+$ channel blockers depress $Na^+$ conduction in ischemic tissue to a much greater extent than in normal tissue. In ischemic tissue, cardiac myocytes are depolarized for a longer period of time. This increase in action potential duration prolongs the inactivation state of the $Na^+$ channels, thereby making the inactivated $Na^+$ channels accessible to $Na^+$ channel blockers for a longer period of time The rate of channel recovery from block is also decreased in depolarized ischemic myocytes because of the prolonged action potential. Thus, *the higher affinity of $Na^+$ channel blockers for open and inactivated states of the channel allows these agents to act preferentially on ischemic tissue and, thereby, to block an arrhythmogenic focus at its source.*

Developing and using effective antiarrhythmic treatments is often complicated by the possibility that the antiarrhythmic agent can also cause arrhythmias. For example, many efforts have been directed at the treatment of re-entry, a mechanism responsible for a large proportion of arrhythmias. One way to treat re-entry is to block action potential propagation. If the retrograde impulse in the re-entrant circuit is *completely extinguished* by an antiarrhythmic agent, then the impulse will be unable to repeatedly depolarize the cardiac tissue in the re-entrant circuit. If the impulse is not completely extinguished, however, then the antiarrhythmic-induced slowing of conduction can actually promote re-entry arrhythmia. The "surviving" impulse may use the original re-entrant pathway to propagate the arrhythmia, or it may find other pathways and create new re-entrant circuits.

## CLASSES OF ANTIARRHYTHMIC AGENTS

Antiarrhythmic agents have traditionally been organized into four classes based on their mechanism of action. Class I antiarrhythmics are $Na^+$ channel blockers; class II antiarrhythmics are β-adrenergic receptor blockers; class III antiarrhythmics are $K^+$ channel blockers; and class IV antiarrhythmics are $Ca^{2+}$ channel blockers. *It is important to realize, however, that many antiarrhythmic agents are not highly selective blockers of $Na^+$, $K^+$, or $Ca^{2+}$ channels; rather, many of these agents block more than one channel type.* This section first presents some useful definitions of common cardiac electrical disturbances (Box 17-2), and then describes the mechanism of drug action for each class of antiarrhythmic agent.

### Class I Antiarrhythmic Agents: Fast $Na^+$ Channel Blockers

*$Na^+$ channel blockers decrease automaticity in SA nodal cells by increasing threshold potential and decreasing the slope of phase 4 depolarization* (Fig. 17-9). When the threshold potential is increased, a greater membrane depolarization is required to reach the potential at which the $Na^+$ channel changes conformation from the resting to the open state. Thus, phase 4 depolarization is extended, and the heart rate is decreased. Clinically, in patients with implanted defibrillators who are treated with $Na^+$ channel blockers, a greater voltage is needed to defibrillate the heart because of an increase in the threshold potential. Therefore, it is important to take into account the effect of $Na^+$ channel blockers when choosing appropriate settings for implanted defibrillators.

In addition to decreasing SA node automaticity, $Na^+$ channel blockers act on ventricular myocytes to decrease reentry. This is achieved mainly by decreasing the upstroke velocity of phase 0 and, for some $Na^+$ channel blockers, by prolonging repolarization (Fig. 17-10). By decreasing phase 0 upstroke velocity, $Na^+$ channel blockers decrease the conduction velocity through cardiac tissue. Ideally, conduction velocity is reduced to such an extent that the propagating wavefront is extinguished before it is able to restimulate myocytes in a re-entrant pathway. However, if conduction velocity is not sufficiently decreased, and the impulse is not extinguished, then the slowed impulse can support re-entry as it reaches cells that are no longer refractory (see above). In addition to decreasing phase 0 upstroke velocity, class IA $Na^+$ channel blockers prolong repolarization. Prolonged repolarization increases the effective refractory period, so that cells in a re-entrant circuit cannot be depolarized by the re-entrant action potential. In summary, *decreasing conduction velocity and increasing the refractory period are the two mechanisms by which $Na^+$ channel blockers decrease the likelihood of re-entry and, thereby, prevent arrhythmia.*

### BOX 17-2. Definitions of Common Cardiac Electrical Disturbances

To appreciate the clinical applications of the various antiarrhythmic agents, it is helpful to understand the basic definitions of terms that describe common electrical abnormalities of the heart.

**Effective refractory period:** The period during which a region of cardiac tissue cannot be excited by an electrical impulse.

**Sinus tachycardia:** The SA node fires between 100 and 180 times per minute, and the EKG shows normal P waves and QRS complexes. Sinus tachycardia can be a normal physiologic response (e.g., during exercise), or a pathologic condition that results from altered SA node automaticity.

**Paroxysmal supraventricular tachycardia (PSVT):** PSVT is characterized by atrial firing rates of 140–250 beats per minute, but it is usually transient and self-limited in nature. In 90% of cases, PSVT is caused by reentry involving the AV node, SA node, or atrial tissue.

**Atrial flutter:** The atrial rate is between 280 and 300 beats per minute, and the EKG shows a rapid, "saw-tooth" appearance of atrial electrical activity. Because the pace of atrial firing is so rapid, some impulses from the atria reach the AV node during its refractory period. These impulses are not transmitted to the ventricles and, therefore, the ventricular rate is slower. The ratio of atrial to ventricular firing rate is usually 2:1.

**Atrial or ventricular fibrillation:** These arrhythmias are characterized by chaotic, re-entrant impulse conduction through the atrium or ventricle. Ventricular fibrillation (VF) is invariably fatal if the arrhythmia is not converted, while atrial fibrillation (AF) can be tolerated for many years.

**Ventricular tachycardia (VT):** A series of three or more ventricular extrasystoles at rates between 100 and 250 beats per minute.

***Torsades de pointes:*** This arrhythmia is often generated by afterdepolarizations in individuals with prolonged QT syndrome. The varying amplitudes of the QRS complex are often described as "twisting of points" along the baseline of an EKG tracing. "*Torsades*" is often transient and self-limited, but can lead to more life-threatening arrhythmias.

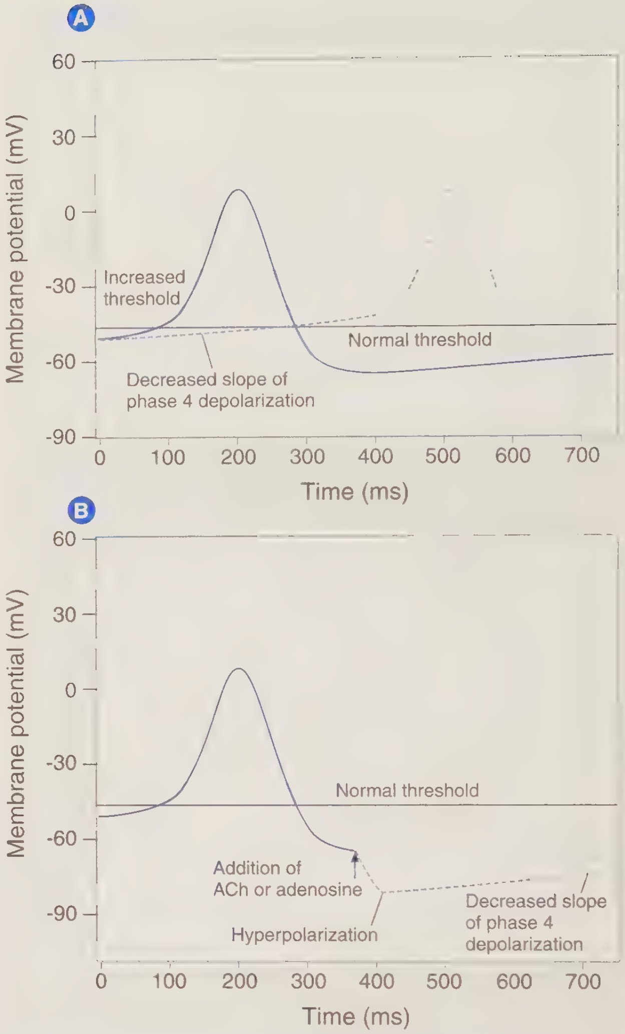


**Figure 17-9. Effects of Class I Antiarrhythmics and Natural Agonists on the SA Node Action Potential.** **A.** The normal SA node action potential is shown as a solid curve. Class I antiarrhythmics ($Na^+$ channel blockers) alter SA node automaticity by affecting two aspects of the SA nodal action potential: (1) the threshold potential is increased; and (2) the slope of phase 4 depolarization is decreased. **B.** Acetylcholine and adenosine slow the SA nodal firing rate by activating $K^+$ channels that hyperpolarize the cell and decrease the slope of phase 4 depolarization.

Although the three subclasses of class I (A, B, and C) antiarrhythmics have similar effects on the action potential in the SA node, there are important differences in their effects on the ventricular action potential.

### *1. Class IA Antiarrhythmics*

Class IA antiarrhythmics exert a moderate block on $Na^+$ channels and prolong the repolarization of both SA nodal cells and ventricular myocytes. By blocking $Na^+$ channels, these agents decrease the phase 0 upstroke velocity, which decreases conduction velocity through the myocardium. In addition, class IA antiarrhythmics block $K^+$ channels and, thereby, reduce the outward $K^+$ current responsible for repolarization of the membrane. This prolongation of repolarization increases the effective refractory period of the cells. Together, the decreased conduction velocity and increased effective refractory period decrease reentry.

**Quinidine,** at one time the most commonly prescribed oral antiarrhythmic agent, is often considered the prototypical drug among the class IA antiarrhythmics. In addition to the pharmacologic actions described above for all class IA antiarrhythmics, quinidine blocks transmitter release from the vagus nerve and, thereby, exerts anticholinergic (vagolytic) effects. *The anticholinergic effect is significant clinically because it can increase conduction velocity through the AV node.* Increased AV nodal conduction can have potentially

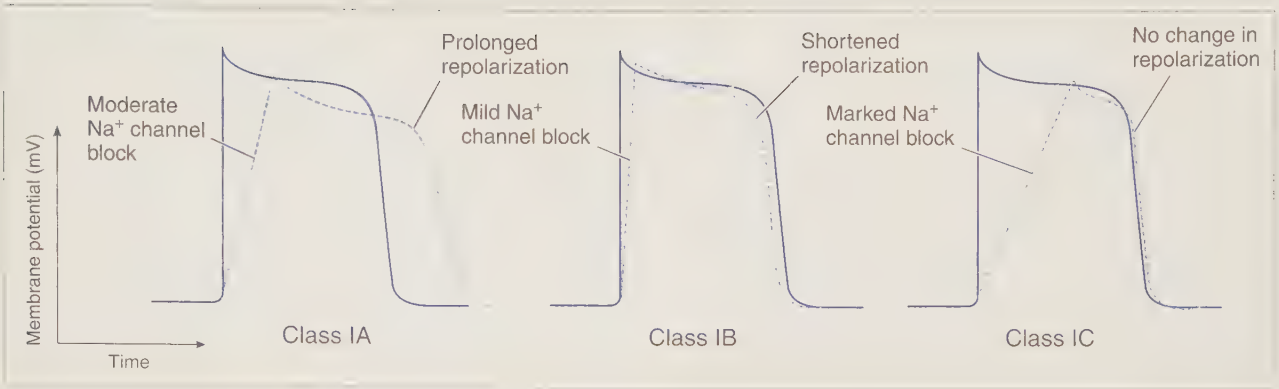


**Figure 17-10. Effects of Class IA, IB, and IC Antiarrhythmics on the Ventricular Action Potential.** Class I antiarrhythmics ($Na^+$ channel blockers) act on ventricular myocytes to decrease re-entry. All subclasses of the class I antiarrhythmics block the $Na^+$ channel to some degree: class IA agents exhibit moderate $Na^+$ channel block, class IB agents rapidly bind to (block) and dissociate from (unblock) $Na^+$ channels, and class IC agents produce marked $Na^+$ channel block. Class IA, IB, and IC agents also differ in the degree to which they affect the duration of the ventricular action potential.

detrimental effects in patients with atrial flutter. Such patients manifest an average atrial firing rate of 280–300 beats per minute. Because some of these impulses reach the AV node while it is still refractory, not all of the impulses are transmitted to the ventricles. Therefore, the atria fire much faster than the ventricles—there is typically a 2:1 or 4:1 ratio of atrial to ventricular firing rates. When quinidine is administered to patients with atrial flutter, the atrial firing rate decreases because of quinidine's pharmacologic action in slowing conduction velocity through the myocardium. At the same time, however, AV nodal conduction velocity increases because of the vagolytic effects of the drug. The increase in AV nodal conduction velocity abolishes the 2:1 or 4:1 ratio of atrial to ventricular firing rates, and a *1:1 ratio* of atrial to ventricular firing rates is established. For example, with an atrial flutter rate of 300 and 2:1 "A–V block," the ventricles are driven at a rate of 150, which most individuals can tolerate. If the flutter rate is slowed to 200 and A–V conduction is enhanced to 1:1, however, the ventricles are driven at a rate of 200, which is usually too fast for effective ventricular pumping. For this reason, an agent that slows AV nodal conduction—such as a β-adrenergic blocker or verapamil (a $Ca^+$ channel blocker)—should be used in conjunction with quinidine to prevent an excessively rapid ventricular response in patients with atrial flutter.

The most common side effects of quinidine are diarrhea, nausea, headache, and dizziness. These side effects make it difficult for patients to tolerate chronic therapy with quinidine. Quinidine is contraindicated in patients with QT prolongation and in patients who are taking medications that predispose to QT prolongation, because of the increased risk of *torsades de pointes.* Relative contraindications to quinidine use include sick sinus syndrome, bundle branch block, myasthenia gravis (because of quinidine's antagonist action at muscarinic receptors), and liver failure.

Quinidine is administered orally and metabolized by cytochrome $P_{450}$ enzymes in the liver. Quinidine increases plasma levels of digoxin (an inotropic agent), most likely by competing for the $P_{450}$ enzymes that are responsible for digoxin metabolism. Because digoxin has a narrow therapeutic index (see Chapter 18), quinidine-induced digoxin toxicity occurs in a significant fraction of patients. The plasma potassium level must be carefully monitored in patients treated with quinidine, because hypokalemia decreases quinidine efficacy, exacerbates QT prolongation and, most importantly, predisposes to *torsades de pointes.* It is hypothesized that *torsades de pointes* is the mechanism most likely responsible for quinidine-induced syncope. Because of quinidine's numerous side effects and contraindications, this drug has largely been replaced by class III agents—such as ibutilide and amiodarone—for the pharmacologic conversion of atrial flutter or fibrillation to normal sinus rhythm.

**Procainamide** is a class IA antiarrhythmic agent that is effective against many types of supraventricular and ventricular arrhythmias. Procainamide is often used in the pharmacologic conversion of new-onset atrial fibrillation to normal sinus rhythm, although with less efficacy than intravenous ibutilide. In the setting of acute myocardial infarction, procainamide can be used safely to decrease the likelihood of re-entrant arrhythmias, even in the presence of decreased cardiac output. Procainamide can be administered by slow intravenous infusion to treat acute ventricular tachycardia.

Unlike quinidine, procainamide has few anticholinergic effects and does not alter plasma levels of digoxin. Procainamide can cause peripheral vasodilation via inhibition of neurotransmission at sympathetic ganglia. With chronic therapy, almost all patients develop a lupus-like syndrome, which can progress to pleural effusions and potentially lethal pericardial tamponade (constriction of the heart caused by accumulation of fluid between the heart and pericardium). Procainamide is acetylated in the liver to N-acetyl-procainamide (NAPA); this active metabolite produces the pure class III antiarrhythmic effects of prolonging the refractory period and lengthening the QT interval.

**Disopyramide** is similar to quinidine in its electrophysiologic and antiarrhythmic effects; the difference between the two drugs lies in their side effects. Disopyramide causes fewer GI problems but has even more profound *anticholinergic* effects than quinidine, producing such side effects as urinary retention and dry mouth. It is contraindicated in patients with obstructive uropathy or glaucoma. Disopyramide is also contraindicated in patients with conduction block between the atria and ventricles and in patients with sinus node dysfunction. Disopyramide has the prominent but unexplained side effect that it depresses cardiac contractility, which has led to its use in the treatment of hypertrophic obstructive cardiomyopathy and neurocardiogenic syncope. Because of its negative inotropic effects, disopyramide is absolutely contraindicated in patients with uncompensated congestive heart failure. Oral disopyramide is approved only for the treatment of life-threatening ventricular arrhythmias; oral or intravenous disopyramide is sometimes used to convert supraventricular tachycardia to normal sinus rhythm. The current trend in the treatment of life-threatening arrhythmias, however, is away from class I antiarrhythmic agents and toward class III agents and electrical devices.

### 2. Class IB Antiarrhythmics

Class IB antiarrhythmics include lidocaine, tocainide, and mexiletine; lidocaine is the prototypical class IB agent. These drugs alter the ventricular action potential by blocking $Na^+$ channels and sometimes by shortening repolarization; the latter effect may be mediated by the drugs' ability to block the few $Na^+$ channels that inactivate late during phase 2 of the cardiac action potential (Fig. 17-10). In comparison to class IA antiarrhythmics, which preferentially bind to open $Na^+$ channels, *class IB drugs bind to both open and inactivated $Na^+$ channels.* The major distinguishing characteristic of the class IB antiarrhythmics is their *fast dissociation* from $Na^+$ channels. Therefore, the more time $Na^+$ channels spend in the open or inactivated state, the more blockade the class IB antiarrhythmics can effect. Because $Na^+$ channels recover quickly from class IB blockade, these drugs are most effective in blocking depolarized or rapidly driven tissues, where there is a higher likelihood of the $Na^+$ channels being in the open or inactivated state. That is, class IB antiarrhythmics exhibit *use-dependent block* in diseased myocardium, where cells fire more frequently; these antiarrhythmics have relatively little effect on normal cardiac tissue.

Myocardial ischemia provides an example of the therapeutic utility of the use-dependent block exerted by class IB antiarrhythmics. The increase in extracellular $H^+$ concentration in ischemic tissue activates membrane pumps that cause an increase in the extracellular $K^+$ concentration. This increase in extracellular $K^+$ shifts $E_K$ to a more depolarized (more positive) value; for example, $E_K$ may shift from –94 mV to –85 mV. The altered electrochemical $K^+$ gradient provides a *smaller* driving force for $K^+$ ions to flow out of cells. Depolarization of the membrane, which results from the decreased outward $K^+$ current, leads to a higher likelihood of firing. Because ischemic cardiac myocytes tend to fire more frequently, the $Na^+$ channels spend more time in the open or inactivated state, serving as a better target for blockade by class IB antiarrhythmics.

**Lidocaine** is the antiarrhythmic agent used most commonly to treat ventricular arrhythmias in emergency situations. This drug is not effective in treating supraventricular arrhythmias. Although lidocaine reduces the incidence of ventricular fibrillation, recent evidence suggests that it also increases mortality rates, presumably because of drug-induced bradycardia and asystole. Therefore, lidocaine is no longer considered a routine prophylactic treatment for prevention of arrhythmia in acute myocardial infarction. Instead, lidocaine is reserved for patients who have ventricular tachyarrhythmias or frequent premature ventricular contractions (PVCs) that compromise the hemodynamic status of the patient. In the opening case, Dr. Johnson manifested a ventricular tachycardia that severely compromised his hemodynamic stability, as evidenced by his low blood pressure (90/60), and he was treated with lidocaine.

Lidocaine has a short plasma half-life—approximately 20 minutes; this is why, in the opening case, Dr. Johnson went into ventricular fibrillation 20 minutes after his IV line was blocked. Lidocaine is de-ethylated in the liver. Its metabolism is governed by two factors, liver blood flow and liver microsomal activity. For patients whose liver blood flow is decreased by old age or heart failure, or whose liver enzymes are acutely inhibited by cimetidine (see Chapter 4, Drug Metabolism), a lower dose of lidocaine should be considered. For patients whose liver enzymes are induced by drugs such as barbiturates, phenytoin, or rifampin, the dose of lidocaine should be increased.

Because lidocaine shortens repolarization, possibly by blocking the few $Na^+$ channels that inactivate late during phase 2 of the cardiac action potential, it does not prolong the QT interval. Therefore, the drug is safe for use in patients with long QT syndrome. However, because lidocaine also blocks $Na^+$ channels in the central nervous system (CNS), it can produce CNS side effects such as confusion, dizziness, and seizures. In addition to its use as an acute intravenous therapy for ventricular arrhythmias, lidocaine is used as a local anesthetic (see Chapter 9, Local Anesthetic Pharmacology, for a detailed discussion).

### 3. Class IC Antiarrhythmics

Class IC antiarrhythmics are the most potent $Na^+$ channel blockers, and they have little or no effect on action potential duration (Fig. 17-10). By markedly decreasing the rate of phase 0 upstroke of ventricular cells, these drugs suppress premature ventricular contractions. Class IC antiarrhythmics also prevent paroxysmal supraventricular tachycardia and atrial fibrillation. However, these drugs have marked depressive effects on cardiac function and, thus, must be used with discretion. In addition, the CAST (Cardiac Arrhythmia Suppression Trial) and other studies have brought attention to the proarrhythmic effects of these agents.

**Flecainide** is the prototypical class IC drug; other members of this class include encainide, moricizine, and propafenone. Flecainide illustrates the principle that antiarrhythmic agents can also cause arrhythmia. When flecainide is administered to patients with pre-existing ventricular tachyarrhythmias and to those with a history of myocardial infarction, it can worsen the arrhythmia even at normal doses. Currently, flecainide is approved for use only in life-threatening situations; for example, when paroxysmal supraventricular or ventricular arrhythmia is unresponsive to other measures. Flecainide is eliminated very slowly from the body; it has a plasma half-life of 12–30 hours. Because of its marked blockade of $Na^+$ channels and its suppressive effects on cardiac function, flecainide use is associated with side effects that include sinus node dysfunction, a marked decrease in conduction velocity, and conduction block.

## Class II Antiarrhythmic Agents: β-Adrenergic Blockers

Class II antiarrhythmic agents are β-adrenergic antagonists (also called β-blockers). These agents act by inhibiting sympathetic input to the pacing regions of the heart. (β-Adrenergic antagonists are more extensively discussed in Chapter 8, Adrenergic Pharmacology, and Chapter 18, Pharmacology of Cardiac Contractility.) Although the heart is capable of beating on its own without innervation from the autonomic nervous system, both sympathetic and parasympathetic fibers innervate the SA node and the AV node and, thereby, alter the rate of automaticity. Sympathetic stimulation releases norepinephrine, which binds to $\beta_1$-adrenergic receptors in the nodal tissues. ($\beta_1$-Adrenergic receptors are the adrenergic subtype preferentially expressed in cardiac tissue.) Activation of $\beta_1$-adrenergic receptors in the SA node triggers an increase in the pacemaker current ($I_f$), which increases the rate of phase 4 depolarization and, consequently, leads to more frequent firing of the node. Stimulation of $\beta_1$-adrenergic receptors in the AV node increases $Ca^{2+}$ and $K^+$ currents and, thereby, increases the conduction velocity and decreases the refractory period of the node.

$\beta_1$-Antagonists block the sympathetic stimulation of $\beta_1$-adrenergic receptors in the SA and AV nodes (Fig. 17-11). The AV node is more sensitive than the SA node to the effects of $\beta_1$ antagonists.

$\beta_1$-Blockers affect the action potentials of the SA and AV nodes by: (1) decreasing the rate of phase 4 depolarization; and (2) prolonging repolarization. Decreasing the rate of phase 4 depolarization results in decreased automaticity and this, in turn, reduces oxygen demand on the heart. Prolonged repolarization at the AV node increases the effective refractory period, which decreases the incidence of re-entry.

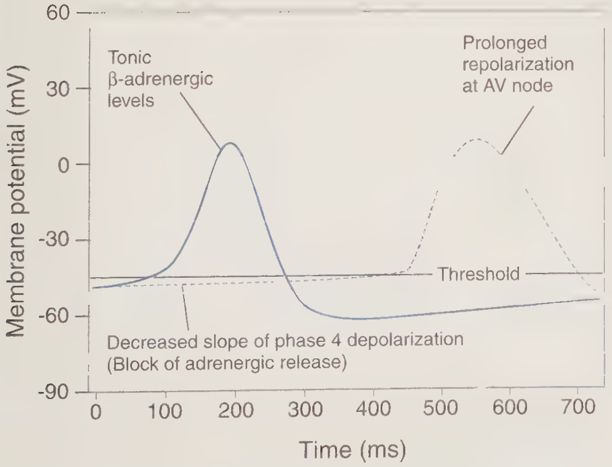


**Figure 17-11. Effects of Class II Antiarrhythmics on Pacemaker Cell Action Potentials.** Class II antiarrhythmics (β-blockers) reverse the tonic sympathetic stimulation of cardiac $\beta_1$-adrenergic receptors. By blocking the adrenergic effects on the SA and AV nodal action potentials, these agents decrease the slope of phase 4 depolarization (especially important at the SA node) and prolong repolarization (especially important at the AV node). These agents are useful in the treatment of supraventricular and ventricular arrhythmias that are precipitated by sympathetic stimulation.

$\beta_1$-Blockers are the most frequently used agents in the treatment of supraventricular and ventricular arrhythmias precipitated by sympathetic stimulation. $\beta_1$-Adrenergic antagonists have been shown to reduce mortality after myocardial infarction, even in patients with relative contraindications to this therapy. Because of their wide spectrum of clinical application and established safety record, β-adrenergic antagonists are the most useful antiarrhythmic agents currently available.

There are several generations of β-blockers, each characterized by slightly different pharmacologic properties. First-generation β-blockers, such as **propranolol,** are nonselective β-adrenergic blockers that antagonize both $\beta_1$-adrenergic and $\beta_2$-adrenergic receptors. They are widely used to treat tachyarrhythmias caused by catecholamine stimulation during exercise or emotional stress. Because propranolol does not prolong repolarization in ventricular tissue, it can be used in patients with long QT syndrome. Second-generation agents, including **atenolol, metoprolol, acebutolol,** and **bisoprolol,** are relatively selective for $\beta_1$-adrenergic receptors when administered in low doses. Third-generation β-blockers cause vasodilation in addition to $\beta_1$-receptor antagonism. **Labetalol** and **carvedilol** induce vasodilation by antagonizing α-adrenergic receptor-mediated vasoconstriction, while **pindolol** is a $\beta_2$-receptor partial agonist.

The different generations of β-blockers produce varying degrees of side effects. Three general mechanisms are responsible for the side effects of β-blockers. First, antagonism at $\beta_2$-adrenergic receptors causes smooth muscle spasm, leading to bronchospasm, cold extremities, and impotence. These side effects are more commonly caused by the nonselective first generation β-blockers. Second, exaggeration of the therapeutic effects of $\beta_1$-receptor antagonism can lead to excessive negative inotropic effects, heart block, and bradycardia. Third, drug penetration into the CNS can produce insomnia and depression.

## Class III Antiarrhythmic Agents: Inhibitors of Repolarization

Class III antiarrhythmic agents block $K^+$ channels. Two types of currents determine the duration of the plateau phase of the cardiac action potential: inward, depolarizing $Ca^{2+}$ currents and outward, hyperpolarizing $K^+$ currents. During a normal action potential, the hyperpolarizing $K^+$ currents eventually dominate, returning the membrane potential to more hyperpolarized values. Larger hyperpolarizing $K^+$ currents shorten plateau duration, returning the membrane potential to its resting value more rapidly, while smaller hyperpolarizing $K^+$ currents lengthen plateau duration and delay return of the membrane potential to its resting value.

When $K^+$ channels are blocked, a smaller hyperpolarizing $K^+$ current is generated. Therefore, $K^+$ channel blockers cause a longer plateau and prolong repolarization (Fig. 17-12). The ability of $K^+$ channel blockers to lengthen plateau duration is responsible for both their pharmacologic uses and their adverse effects. On the beneficial side, prolongation of the plateau duration increases the effective refractory period, which, in turn, decreases the incidence of re-entry. On the toxic side, prolongation of the plateau duration increases the likelihood of developing early afterdepolarizations and *torsades de pointes.*

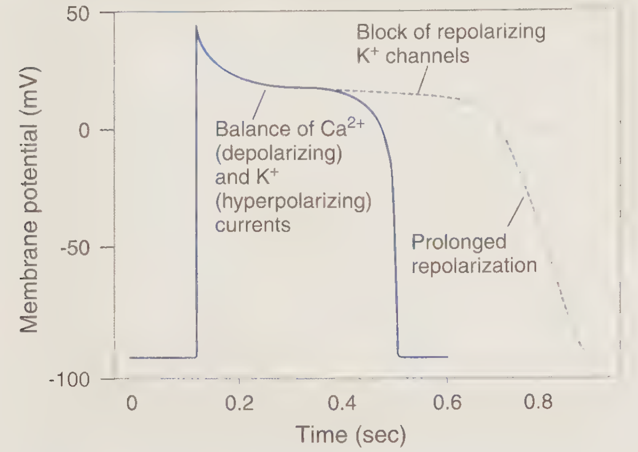


**Figure 17-12. Effects of Class III Antiarrhythmics on the Ventricular Action Potential.** Class III antiarrhythmics ($K^-$ channel blockers) decrease the magnitude of the repolarizing $K^+$ currents during phase 2 of the action potential and, thereby, prolong action potential duration. This prolongation of the plateau phase decreases re-entry, but it can also predispose to early afterdepolarizations.

With the exception of amiodarone, $K^+$ channel blockers also exhibit the undesirable property of "reverse use-dependency:" action potential prolongation is most pronounced at slow rates (undesirable) and least pronounced at fast rates (desirable). $K^+$ channel blockers have little or no effect on the upstroke phase or conduction velocity of the impulse.

**Ibutilide** is a class III agent that prolongs repolarization by inhibiting the delayed rectifier $K^+$ current. This agent also enhances a slow inward $Na^+$ current that further prolongs repolarization. Ibutilide is used to terminate atrial fibrillation and flutter. The major side effect of ibutilide results from its prolongation of the QT interval; the serious arrhythmia *torsades de pointes* may result, requiring cardioversion in almost 2% of patients taking the drug. Ibutilide is generally not administered to patients with pre-existing prolonged QT syndrome.

**Sotalol** is a mixed class II and class III antiarrhythmic agent. This drug nonselectively antagonizes β-adrenergic receptors (class II action), and it also prolongs action potential duration by blocking $K^+$ channels (class III action). Sotalol exists in two isomeric forms, the *l*-isomer and *d*-isomer. While the two isomeric forms are equipotent in blocking $K^+$ channels, the *l*-form is a more potent β-blocker. Sotalol is used to treat severe ventricular arrhythmias, especially in patients who cannot tolerate the side effects of amiodarone. Sotalol is also used to prevent recurrent atrial flutter or fibrillation and, thereby, to maintain normal sinus rhythm. As a β-blocker, sotalol can cause fatigue and bradycardia; like other class III antiarrhythmic agents, it can induce *torsades de pointes.*

**Bretylium** acts as both an antihypertensive agent and a class III antiarrhythmic agent. Like guanethidine (see Chapter 8), this drug is concentrated in the terminals of sympathetic neurons, causing an initial release of norepinephrine but then inhibiting further release of norepinephrine. In effect, bretylium performs a "chemical sympathectomy" and, thereby, exerts an antihypertensive effect. Bretylium also increases the action potential duration in normal and ischemic cardiac cells. Its sites of antiarrhythmic activity are mainly in the Purkinje fibers and secondarily in ventricular myocytes; it has no effect on atrial tissue. Bretylium is indicated only in patients with recurrent ventricular tachycardia or fibrillation after lidocaine and defibrillation measures have failed. Because of its sympatholytic effects, bretylium can cause marked hypotension.

**Amiodarone** is mainly a class III antiarrhythmic agent, but it also acts as a class I, class II, and class IV antiarrhythmic. The ability of amiodarone to exert such a diversity of effects can be explained by its mechanism of action: *alteration of the lipid membrane in which ion channels and receptors are located.* In all cardiac tissues, amiodarone lengthens the effective refractory period by blocking the $K^+$ channels responsible for repolarization; this prolongation of action potential duration decreases re-entry. As a potent class I agent, amiodarone blocks $Na^+$ channels and, thereby, decreases the rate of firing in pacemaker cells; it exhibits use-dependent $Na^+$ channel blockade by binding preferentially to channels in the inactivated conformation. Amiodarone exerts class II antiarrhythmic activity by noncompetitively antagonizing α-adrenergic and β-adrenergic receptors. Finally, as a $Ca^{2+}$ channel blocker (class IV), amiodarone can cause significant AV nodal block and bradycardia, although its use is fortunately associated with a relatively low incidence of *torsades de pointes.*

In recent years, the results of several clinical trials have caused the popularity of amiodarone to increase from a last-resort agent to one that is frequently used to treat life-threatening arrhythmias. The clinical indications for amiodarone are highly dose-dependent. Because high-dose amiodarone has substantial toxicity, the drug is used at full dose only in patients with hemodynamically unstable ventricular tachycardia or fibrillation after other antiarrhythmic agents have failed. At reduced doses, however, amiodarone is one of the most effective agents in preventing serious ventricular arrhythmias in patients with congestive heart failure and/or a history of recent myocardial infarction. Amiodarone is also highly effective in the prevention of recurrent paroxysmal atrial fibrillation or flutter.

The wide spectrum of action of amiodarone is accompanied by a panoply of serious side effects when the drug is used for long periods or in high doses. These side effects include cardiac, pulmonary, thyroid, hepatic, neurologic, and idiosyncratic complications (Table 17-2). In the heart, amiodarone can decrease AV or SA node function by inhibiting $Ca^{2+}$ channels. As an α-adrenergic antagonist, amiodarone can cause hypotension. Amiodarone can exert a negative inotropic effect by inhibiting β-adrenergic receptors, especially when the drug is used chronically. Severe pulmonary complications can occur in patients taking high doses of amiodarone (400 mg daily). Pneumonitis leading to pulmonary fibrosis is the most dreaded of all complications associated with amiodarone use. Fortunately, such complications occur rarely in patients taking prophylactic doses (200 mg daily) for prevention of ventricular or atrial arrhythmias. Because of its structural similarity to thyroxine, amiodarone affects thyroid hormone metabolism by inhibiting peripheral conversion of thyroxine ($T_4$) to triiodothyronine ($T_3$). Both hyperthyroidism and hypothyroidism can occur as a consequence of this dysregulation of thyroid hormone metabolism (see Chapter 25, Pharmacology of the

**TABLE 17-2** Major Side Effects of Amiodarone, Particularly at High Doses

| CATEGORY | SIDE EFFECT |
|---|---|
| Cardiovascular | ↓AV or SA node function<br>↓ Cardiac contractility<br>Hypotension |
| Pulmonary | Pneumonitis leading to pulmonary fibrosis |
| Thyroid | Hyperthyroidism or hypothyroidism |
| Hepatic | Elevated liver enzymes (LFTs) |
| Neurologic | Peripheral neuropathy, headache, ataxia, tremors |
| Other | Corneal microdeposits<br>Testicular dysfunction<br>Skin discoloration |

Thyroid Gland). Ten to 20% of patients taking amiodarone manifest an abnormal increase in liver enzymes, although this side effect is reversible when the dose of the drug is reduced. Neurologic symptoms can include peripheral neuropathy, headache, ataxia, and tremors. Therefore, patients taking amiodarone should be monitored periodically for abnormal pulmonary, thyroid, and liver function.

Amiodarone is contraindicated in patients with cardiogenic shock, second-degree or third-degree heart block, or severe SA node dysfunction with marked sinus bradycardia or syncope.

## Class IV Antiarrhythmic Agents: $Ca^{2+}$ Channel Blockers

Drugs that block cardiac $Ca^{2+}$ channels act preferentially on *SA and AV nodal tissues,* because these pacemaker tissues depend on $Ca^{2+}$ currents for the depolarization phase of the action potential (Fig. 17-2). In contrast, $Ca^{2+}$ channel blockers have little effect on fast $Na^+$ channel-dependent tissues, such as Purkinje fibers and atrial and ventricular muscle. $Ca^{2+}$ channel blockade has two major effects on the action potentials of the SA and AV nodes. First, as noted above, $Ca^{2+}$ channel blockers slow the rise of the action potential upstroke (phase 0) in both the SA and AV nodes. Second, and somewhat paradoxically, these agents prolong repolarization of the AV node (Fig. 17-13). The effects of $Ca^{2+}$ channel blockers on the electrophysiology of the AV node can be summarized as follows: *slowing the action potential upstroke leads to slowed conduction velocity through the AV node, while prolonged repolarization increases the effective refractory period of the AV node.* Together, these two actions are very effective in treating re-entrant arrhythmias in which the AV node is a component of the reentry circuit. (Refer to Chapter 20 for a more extended discussion of $Ca^{2+}$ channel blockers.)

Because different tissues express different subtypes of $Ca^{2+}$ channels, and different subclasses of $Ca^{2+}$ channel blockers interact preferentially with different $Ca^{2+}$ channel subtypes, the various $Ca^{2+}$ channel blockers have differential effects in different tissues. Dihydropyridines (such as **nifedipine**) have relatively more effect on the $Ca^{2+}$ current in *vascular smooth muscle,* while **verapamil** and **diltiazem** are more effective in *cardiac tissues.* Verapamil and diltiazem are used to treat re-entrant paroxysmal supraventricular tachycardias, because these are often re-entrant arrhythmias that involve the AV node. Verapamil and diltiazem are *rarely* used in ventricular tachycardia. In fact, the only indications for these agents in ventricular arrhythmias are idiopathic right ventricular outflow tract tachycardia and fascicular tachycardias. Verapamil is also used to treat hypertension and vasospastic (Prinzmetal) angina. Class IV agents can cause AV nodal block by excessively reducing the conduction velocity. Administration of intravenous verapamil to patients taking β-blockers can precipitate severe congestive heart failure and lead to irreversible electromechanical dissociation. Verapamil and diltiazem increase plasma digoxin levels by competing with the latter drug for renal excretion.

## Other Antiarrhythmic Agents: Adenosine and Potassium

Adenosine and potassium, while not considered classical antiarrhythmic agents, have important effects on cardiac electrophysiology. Adenosine can be used to treat arrhythmias that involve aberrant AV nodal conduction; whereas, physiologic concentrations of $K^+$ must be maintained to prevent arrhythmias.

### *1. Adenosine*

The nucleoside **adenosine** is naturally present throughout the body. By stimulating the P1 class of purinergic receptors, adenosine opens a G protein-coupled $K^+$ channel and, thereby, inhibits SA nodal, atrial, and AV nodal conduction. The AV

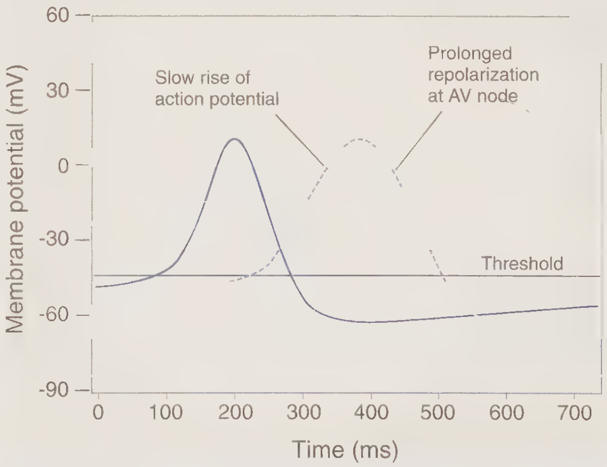


**Figure 17-13. Effects of Class IV Antiarrhythmics on Pacemaker Cell Action Potentials.** Class IV antiarrhythmics ($Ca^{2+}$ channel blockers) exert two effects on the action potentials of SA and AV nodal cells. First, these agents slow the rise of the upstroke (phase 0) of both SA and AV nodal action potentials. Second, by a more complicated mechanism, these agents prolong repolarization of AV nodal cells. Because both of these actions prolong AV nodal conduction, class IV antiarrhythmics are useful in the treatment of arrhythmias that involve re-entry through the AV node. Excessive doses of $Ca^{2+}$ channel blockers can prolong AV nodal conduction to such an extent that heart block results.

node is more sensitive than the SA node to the effects of adenosine. Adenosine also inhibits cAMP-induced $Ca^{2+}$ influx and, thereby, suppresses $Ca^{2+}$-dependent action potentials. Adenosine has a plasma half-life of less than 10 seconds and is often used as the first-line agent for converting narrow-complex paroxysmal supraventricular tachycardia to normal sinus rhythm. For this indication, it is efficacious in 90% of cases. Most side effects of adenosine are transient, including headache, flushing, chest pain, and excessive AV or SA nodal inhibition. Adenosine can also cause bronchoconstriction lasting for up to 30 minutes in asthmatic patients. In 65% of patients, a *transient* new arrhythmia occurs at the onset of adenosine administration.

### 2. Potassium

Hypokalemia and hyperkalemia can both be arrhythmogenic and, therefore, the plasma $K^+$ level must be carefully monitored. Hypokalemia can cause early afterdepolarizations, delayed afterdepolarizations, and ectopic beats in nonpacemaker cells. Hyperkalemia can lead to a marked slowing in conduction velocity, because increased extracellular $K^+$ concentrations lower $E_K$ and, thus, depolarize membranes. The life-threatening cardiac effects of hyperkalemia are the primary impetus for initiating dialysis in patients with renal failure.

Potassium is an antiarrhythmic agent in two ways. First, serum $K^+$ levels outside the physiologic range (3.5–5 mM) can be an important factor in initiating and maintaining arrhythmias. Correction of hypokalemia or hyperkalemia alone can be sufficient to terminate some arrhythmias. Second, it is possible to use supraphysiologic or subphysiologic serum $K^+$ concentrations in an attempt to terminate arrhythmias. This is *rarely* done in clinical practice, however, because it is difficult to change $K^+$ levels reliably, and exogenously induced changes in $K^+$ levels are rapidly corrected by renal mechanisms.

## Conclusion and Future Directions

Cardiac arrhythmias arise from defects in impulse formation, defects in impulse conduction, or a combination of the two mechanisms. Because derangements in ion conductance lead to arrhythmias, antiarrhythmic agents act by directly or indirectly altering the conformational states of ion channels and, thereby, changing the membrane permeability to ions. The pharmacologic property of use-dependent ion channel blockade allows many antiarrhythmic agents to target diseased cardiac tissues preferentially, based on the altered electrophysiology of these tissues. In general, class I antiarrhythmics block $Na^+$ channels; class II antiarrhythmics (β-blockers) inhibit sympathetic stimulation, and thereby decrease automaticity; class III agents block $K^+$ channels; and class IV agents block $Ca^{2+}$ channels. Despite continuing developments in antiarrhythmic drugs, the paradox still exists that antiarrhythmic drugs can generate arrhythmias. Nonetheless, judicious use of antiarrhythmic agents can reduce mortality in certain clinical circumstances, and careful tailoring of a drug regimen to the individual patient's clinical status can reduce side effects.

**TABLE 17-3** Molecular Identity of Cardiac Ion Currents

| ION CURRENT | CHANNEL PROTEIN |
|---|---|
| $I_{Na}$ | $Na_V1.5$ |
| $I_{Ca.L}$ (dihydropyridine-sensitive) | $Ca_V1.2$ |
| $I_{Ca.T}$ | $Ca_V3.1$ |
| $I_f$ | HCN2, HCN4 |
| $I_{to}$ | $K_V4.3$ |
| $I_{Ks^*}$ | $K_V7.1$ (KvLQT1) |
| $I_{Kr^*}$ | $K_V11$, HERG |
| $I_{K1}$ | Kir2.1 (inward rectifier) |
| $I_{KACh}$ | Kir3.1+Kir3.4 (G protein-gated) |

*Collectively referred to as $I_K$.

The most important new directions in the pharmacology of cardiac rhythm involve identifying specific genes for ion channels in the human heart (Table 17-3). Currently, animal models are used for the majority of ion channel research; comparatively little is known about the clinical pharmacology of ion channel expression in humans. With the mouse and human genomes now completely sequenced, researchers will be able to investigate the possibility that these molecules can serve as selective targets for new therapeutic agents. The identification of ion channel gene expression in the various tissues of the human heart (SA node, AV node, atrial conduction pathways, endocardium, ventricular conduction pathways, etc.), during development, and in response to injury may provide specific targets that are not now known. Many of the genes are likely to encode channels that form heteromultimers of various kinds, and there are likely to be many genetic variants within the population.

Over the next 10 years, it is expected that the human cardiac channels, their precise tissue distributions, and their combinations as heteromultimers will be defined. The enormous complexity will likely represent a boon to drug development because it will allow many more targeting strategies to be employed. In parallel, the development of implantable computers, stimulators, and defibrillators will comprise an alternative strategy to prevent or terminate arrhythmias.

## Suggested Readings

Ackerman MJ, Clapham DE. Normal cardiac electrophysiology: Understanding the action potential in human heart. In: Chien KR, Ed. Molecular Basis of Cardiovascular Disease. Philadelphia: WB Saunders, 1999:281–301.

Ackerman MJ, Clapham DE. Ion channels—Basic science and clinical disease. N Engl J Med 1997;336:1575–1586. (*Broad review of ion channels.*)

Grace A, Camm AJ. Drug therapy: Quinidine. N Engl J Med 1998; 338:35–45. (*Focused discussion on quinidine efficacy and adverse effects.*)

Katz A. Mechanisms of disease: Cardiac ion channels. N Engl J Med 1993;328:1244–1251. (*Molecular basis of cardiac arrhythmias.*)

Kowey PR, Marinchak RA, Rials SJ, Bharucha DB. Classification and pharmacology of antiarrhythmic drugs. Am Heart J 2000; 140:12–20. (*Updated synopsis of antiarrhythmic drugs.*)

Lilly L, ed. Pathophysiology of Heart Disease. Baltimore, MD: Lippincott Williams & Wilkins, 1998. (*More details on the underlying pathophysiology of cardiac arrhythmias.*)

## Drug Summary Table Pharmacology of Cardiac Rhythm

| Drug | [illegible] | [illegible] | [illegible] | [illegible] |
|---|---|---|---|---|
| **CLASS IA ANTIARRHYTHMICS**<br>*Mechanism—Moderate sodium channel blockade, prolongation of repolarization* | | | | |
| **Quinidine** | Atrial and ventricular arrhythmias | Cinchonism, headache, tinnitus, hemolytic anemia, thrombocytopenia, *torsades de pointes,* syncope | Preexisting prolonged QT interval | Not well tolerated by patients due to its numerous side effects, including the serious side effect of syncope |
| **Procainamide** | Atrial and ventricular arrhythmias | Reversible lupus-like syndrome | | Procainamide is metabolized to NAPA, a class III antiarrhythmic agent |
| **Disopyramide** | Atrial and ventricular arrhythmias | Fewer GI side effects but greater anticholinergic side effects (urinary retention, dry mouth) | Uncompensated congestive heart failure | |
| **CLASS IB ANTIARRHYTHMICS**<br>*Mechanism—Mild sodium channel blockade, shortening of repolarization* | | | | |
| **Lidocaine**<br>**Mexiletine**<br>**Tocainide** | Acute ventricular arrhythmias, digitalis-induced arrhythmias | Confusion, dizziness, seizures | | Intravenous only<br>Lidocaine also used as a local anesthetic |
| **CLASS IC ANTIARRHYTHMICS**<br>*Mechanism—Strong sodium channel blockade* | | | | |
| **Flecainide**<br>**Encainide**<br>**Propafenone** | Last resort to treat refractory VT that progresses to VF and intractable SVT | Proarrhythmic | | Due to serious proarrhythmic effects, used only as a last resort |
| **CLASS II ANTIARRHYTHMICS**<br>*Mechanism—β-adrenergic receptor blockade* | | | | |
| **Propranolol**<br>**Esmolol**<br>**Metoprolol**<br>**Atenolol** | VT and SVT | Bronchospasm, impotence, bradycardia, heart block, insomnia, depression | Nonselective β-blockers are relatively contraindicated in asthmatics | May mask signs of hypoglycemia<br>Also see Chapter 8 |

*(Continued)*

**Drug Summary Table** **Pharmacology of Cardiac Rhythm** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **CLASS III ANTIARRHYTHMICS**<br>*Mechanism—Prolongation of action potential, mainly through potassium channel blockade* | | | | |
| **Ibutilide** | Atrial fibrillation and flutter | Prolongs QT interval and predisposes to *torsades de pointes* | Preexisting prolonged QT interval | |
| **Sotalol** | Supraventricular and ventricular tachycardia | Predisposes to *torsades de pointes,* excessive β blockade | Preexisting prolonged QT interval | Also a nonselective β-adrenergic receptor antagonist |
| **Bretylium** | Life-threatening VF and VT unresponsive to lidocaine and defibrillation | Orthostatic hypotension, new arrhythmias | | Also an antihypertensive agent |
| **Amiodarone** | Atrial fibrillation, hypertrophic cardiomyopathy, SVT<br>Chemical cardioversion of malignant ventricular arrhythmia, VF, pulseless VT | Pulmonary fibrosis, hepatotoxicity, thyroid dysregulation, corneal deposits, skin deposits leading to photodermatitis, neurological effects | Severe SA node disease, second or third degree AV block, bradycardia-induced syncope | Monitor pulmonary, liver, and thyroid functions |
| **CLASS IV ANTIARRHYTHMICS**<br>*Mechanism—Calcium channel blockade* | | | | |
| **Verapamil**<br>**Diltiazem** | Angina, hypertension, atrial fibrillation and flutter<br>Verapamil is also used in SVT | CHF (especially verapamil), AV block, sinus node depression, constipation, flushing, edema | LV dysfunction, second or third degree AV block, atrial fib/flutter with bypass tract, sick sinus syndrome | |
| **ADENOSINE**<br>*Mechanism—Enhances K⁺ conductance and inhibits Ca²⁺ influx in SA and AV nodal cells* | | | | |
| **Adenosine** | PSVT conversion | Arrhythmia | | |

# 18

# Pharmacology of Cardiac Contractility

Christopher W. Myers and Thomas P. Rocco

**Introduction**
**Case**
**Physiology of Cardiac Contraction**
  Myocyte Anatomy
  Myocyte Contraction
  Regulation of Contractility
    *The Sodium Pump and the Sodium–Calcium Exchanger*
    *Calcium Storage and Release*
    *Sensitivity of Contractile Proteins to Calcium*
    *cAMP*
**Pathophysiology**
  Cellular Pathophysiology of Contractile Dysfunction
**Pharmacologic Classes and Agents**
  Cardiac Glycosides
    *Digoxin*
    *Digitoxin*
  β-Adrenergic Receptor Agonists
    *Dopamine*
    *Dobutamine*
    *Epinephrine*
    *Norepinephrine*
  Phosphodiesterase (PDE) Inhibitors
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

In 1785, Dr. William Withering described the benefits of a preparation from the foxglove plant called **digitalis.** He used this preparation to treat patients suffering from "dropsy," a condition in which accumulation of extravascular fluid leads to dyspnea (difficulty breathing) and peripheral edema. These symptoms are now recognized as characteristic manifestations of **congestive heart failure (CHF)** caused by systolic dysfunction of the left ventricle. In this condition, the left ventricle (LV) is unable to maintain adequate stroke volume despite normal filling volumes. The left ventricular end-diastolic volume is increased in an effort to preserve stroke output, and this does result in increased stroke volume. Beyond a certain end-diastolic volume, however, LV diastolic pressures begin to increase, often precipitously. These increased LV diastolic pressures result in increased left atrial and pulmonary capillary pressures which, in turn, lead to interstitial and alveolar edema and to increased right heart and pulmonary artery pressures. The elevated right heart pressures cause systemic venous hypertension and peripheral edema.

Dr. Withering's use of digitalis presaged the current use of **digoxin,** a member of the **cardiac glycoside** family of drugs, to treat conditions in which myocardial contractility is impaired. Cardiac glycosides are **positive inotropes,** that is, agents that increase the contractile force of cardiac myocytes. Since the advent of digitalis, elucidation of the cellular mechanism of cardiac contraction has facilitated the development of other inotropic agents. After reviewing the physiology of cardiac contraction and the cellular pathophysiology of contractile dysfunction, this chapter describes three families of positive inotropic drugs that are either used clinically or under investigation in clinical trials. An integrated discussion of therapeutic strategies for CHF can be found in Chapter 23, Integrative Cardiovascular Pharmacology.

## Case

GW, a 68-year-old man with known congestive heart failure, is admitted to the hospital following abrupt loss of consciousness. The patient's cardiac history is notable for two prior myocardial infarctions, the second occurring about 2 years ago. Since the second infarction, the patient has had significant limitation of his exercise capacity and documented nonsustained ventricular tachycardia. A two-dimensional echocardiogram has been notable for a left ventricular ejection fraction of 25% (normal, > 55%) and moderate mitral valve regurgitation. GW has been treated with aspirin, carvedilol (a β-blocker), captopril (an angiotensin converting enzyme inhibitor), digoxin (a cardiac glycoside), furosemide (a loop diuretic), and amiodarone (a class III antiarrhythmic).

Approximately 6 months ago, the patient was diagnosed with a primary lung malignancy. An oncologist has been treating him with a combination of radiation therapy and chemotherapy, and GW has lost ~20% of his body weight (from 200 lb to 158 lb). Over the past week, he has experienced recurrent nausea, vomiting, and diarrhea. He has had little appetite and has not been drinking normally, although he has faithfully taken his medications. Physical examination in the emergency room is notable for a blood pressure of 90/50 mm Hg and a heart rate of 40 beats/min. Laboratory tests show serum $Na^+$ 148 mEq/L (normal, 135–145), BUN 56 mg/dl (normal, 7–18), $K^+$ 2.9 mEq/L (normal, 3.5–5.1), and creatinine 2.8 mg/dL (normal, 0.6–1.2). Additionally, serum digoxin is 5.8 ng/mL (therapeutic concentration, typically ~1 ng/mL). Twelve-lead ECG shows atrial fibrillation with third degree (complete) atrioventricular (AV) block and a ventricular escape rhythm at 40 beats/min.

Based on these findings, which indicate digoxin toxicity, oral $K^+$ is administered to increase GW's serum potassium level. He is monitored in the hospital for 5 days, and his oral digoxin dose is decreased until his serum digoxin level falls back into the therapeutic range.

## QUESTIONS

■ **1.** What is the mechanism of action of digoxin?

■ **2.** What is GW's major clinical manifestation of digoxin toxicity?

■ **3.** What factors (including drug interactions) have contributed to digoxin toxicity in this patient?

■ **4.** Why is GW being treated with a β-blocker and a positive inotrope at the same time?

## PHYSIOLOGY OF CARDIAC CONTRACTION

The heart is responsible for receiving deoxygenated blood from the periphery, transporting this blood through the pulmonary circulation (where the hemoglobin is reoxygenated), and distributing this oxygenated blood to peripheral tissues. To accomplish the latter task, the left ventricle must develop tension to overcome the impedance to ejection that resides in the peripheral circulation. The relationship between the tension generated during the systolic phase of the cardiac cycle and the extent of left ventricular filling is referred to as the **contractile state** of the myocardium or **myocardial contractility.** Together with **preload** (intraventricular blood volume), **afterload** (the resistance against which the left ventricle ejects), and **heart rate,** myocardial contractility is an important determinant of cardiac output. Although cardiac pump performance at the organ level has been a central concern of cardiac physiologists for many years, research directed at the cellular and molecular mechanisms of cardiac contraction is now intensive, including studies at the level of genetic regulation. This section reviews the basic interactions among the ion channels, signaling pathways, and contractile and regulatory proteins that underlie contraction of the normal myocardium.

### MYOCYTE ANATOMY

Like skeletal muscle, cardiac muscle contracts when action potentials depolarize the plasma membranes of cardiac muscle cells. This process of **excitation–contraction (EC) coupling,** in which the intracellular machinery transduces an electrochemical signal into mechanical force, involves the following cascade of events: voltage-gated calcium channels open, intracellular calcium increases, contractile proteins activate, and actin–myosin interactions shorten the myocyte.

The cellular anatomy of ventricular myocytes reflects the function of these cells to execute and regulate contraction (Fig. 18-1). Specialized components of the ventricular myocyte include the **sarcolemma,** or myocyte plasma membrane; the **sarcoplasmic reticulum (SR),** a large internal membrane system that encircles the myofibrils; and the **myofibrils** themselves. Myofibrils are rope-like units containing precisely organized contractile proteins; the coordinated interaction of these proteins is responsible for the physical shortening of the cardiac muscle. These anatomic specializations are illustrated in Figures 18-1 and 18-2, and summarized in Table 18-1.

### MYOCYTE CONTRACTION

Increased cytosolic $Ca^{2+}$ initiates myocyte contraction and is the link between excitation and contraction. During the ventricular action potential (see Chapter 17, Pharmacology of Cardiac Rhythm), increased $Ca^{2+}$ flux through L-type $Ca^{2+}$ channels in the sarcolemma causes an increase in the cytosolic $Ca^{2+}$ concentration. This "trigger calcium" stimulates the ryanodine receptor in the SR membrane, causing release of stored $Ca^{2+}$ from the sarcoplasmic reticulum into the cytosol. When the $Ca^{2+}$ concentration in the cytoplasm reaches approximately $10^{-5}$ M, calcium binds to troponin C and induces a conformational change in tropomyosin that releases the inhibitory protein troponin I. This release of troponin I exposes an interaction site for myosin on the actin filament, and the binding of myosin to actin initiates the contraction cycle.

Figure 18-2 illustrates the cycle by which actin–myosin interactions physically shorten the sarcomere. Each myosin filament is studded with protruding flexible heads that form reversible cross bridges with the actin filaments. Formation of actin–myosin cross bridges, bending of the myosin heads at their flexible hinges, and detachment of the cross bridges allows the myosin filament to "walk up" the actin filament in both directions and, thereby, to pull the two ends of the sarcomere together.

The normal function of the sarcomeric cross bridge cycle is critically dependent on ATP. The ATP hydrolysis (ATPase) activity of myosin provides the energy used both to drive contraction and to reset the contractile proteins, leading to relaxation. If an insufficient amount of ATP is available for cross bridge cycling, then the myosin and actin remain "locked" in the associated state and the myocardium is unable to relax. This ATP-dependence explains the profound impact of ischemia on both systolic contraction (because the contraction cycle cannot proceed) and diastolic relaxation

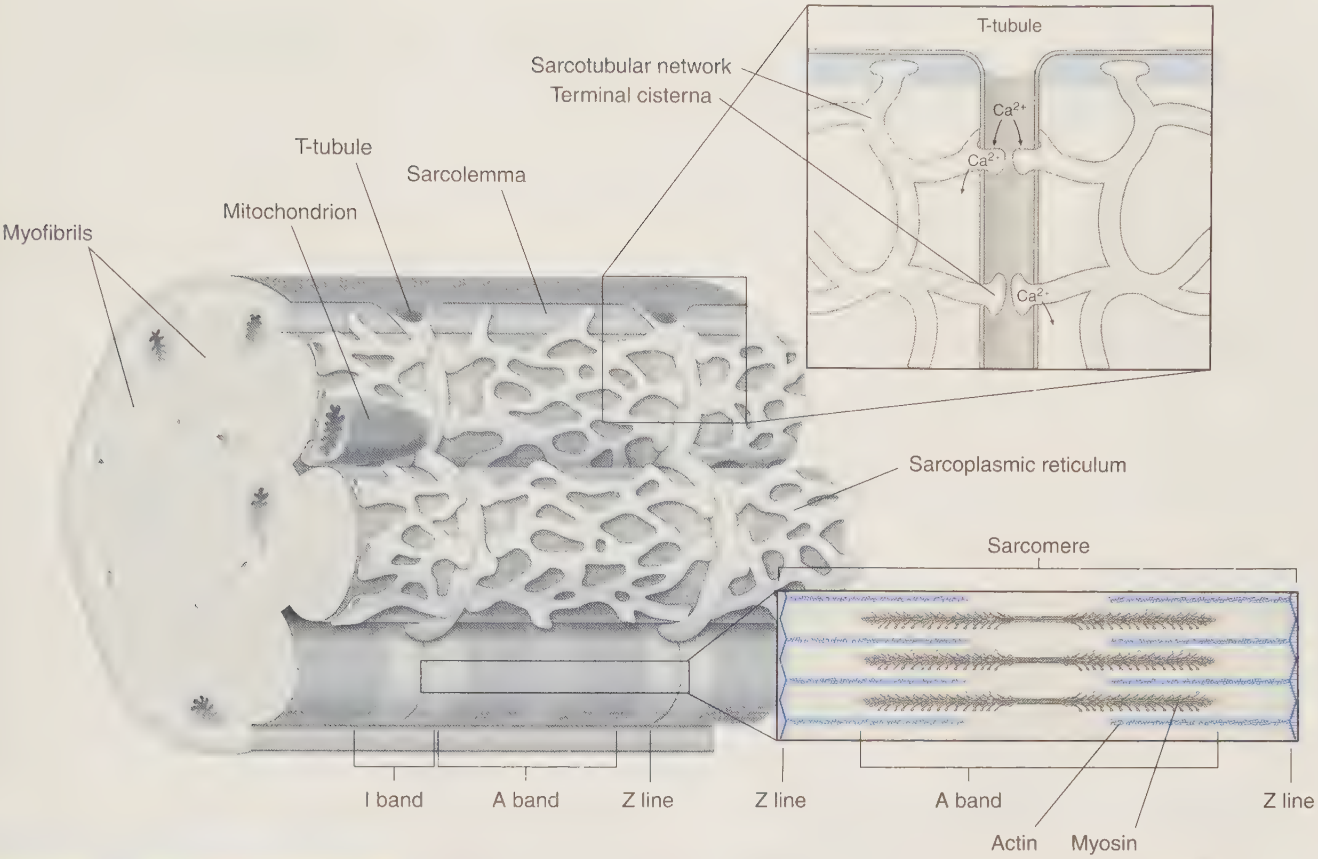


**Figure 18-1. Cardiac Myocyte Structure.** Each cardiac myocyte contains myofibrils and mitochondria surrounded by a specialized plasma membrane termed the sarcolemma. Invaginations of the sarcolemma, called T-tubules, provide conduits for $Ca^{2+}$ influx. Within the cell, an extensive sarcoplasmic reticulum stores $Ca^{2+}$ for use in contraction. Extracellular $Ca^{2+}$ enters through the sarcolemma and its T-tubules during phase 2 of the action potential. This trigger $Ca^{2+}$ binds to channels on the sarcoplasmic reticulum membrane, causing release into the cytosol of a large pool of so-called activation $Ca^{2+}$. Increased cytosolic $Ca^{2+}$ initiates myofibril contraction. The sarcomere is the functional unit of the myofibril. Each sarcomere consists of interdigitating bands of actin and myosin. These bands form distinctive structures under the electron microscope. The A bands correspond to regions of overlapping actin and myosin. The Z lines demarcate the borders of each sarcomere. The I bands span neighboring sarcomeres and correspond to regions of actin without overlapping myosin. During cardiac myocyte contraction, the I bands become shorter (i.e., the Z lines approach one another), but the A bands maintain a constant length.

(because the actin and myosin cannot dissociate) of the myocardium.

The organization of the sarcomere and the physical mechanism of contraction explain the fundamental relationship between muscle length and tension development known as the **Starling relationship.** Increased stretch (length) of the muscle exposes additional sites for calcium binding and for actin–myosin interaction; increased stretch also affects greater release of calcium from the SR. These cellular events provide the mechanistic explanation for the Frank–Starling relationship, which is a fundamental descriptor of cardiac performance: *an increase in the end-diastolic volume of the left ventricle leads to an increase in stroke volume.* Chapter 23 describes organ-level implications of the **Frank–Starling law** in more detail.

In recent years, assays of cardiac troponin levels in the serum have been shown to be sensitive and specific indicators of myocardial injury or infarction. Normal plasma contains negligible levels of cardiac troponin, but these levels rise dramatically after myocardial injury or infarction; the elevation begins as soon as 6 hours after a myocardial infarction, and can persist for 10 days.

## REGULATION OF CONTRACTILITY

A number of control mechanisms regulate calcium cycling in cardiac myocytes (Table 18-2). The cystolic calcium concentration depends on the entry and exit of calcium across the sarcolemma and the release and uptake of calcium by the sarcoplasmic reticulum. The ion channels that mediate these calcium fluxes must function appropriately if the cell is to contract normally. Neurohumoral influences, especially the β-adrenergic signaling pathway, constitute another set of regulatory control mechanisms.

### The Sodium Pump and the Sodium–Calcium Exchanger

In the sarcolemma, the three key proteins involved in calcium regulation are the $Na^+/K^+$-ATPase, hereafter referred to as the **sodium pump,** the **sodium–calcium exchanger,** and the calcium–ATPase or **calcium pump** (Fig. 18-3). The activity of the sodium pump is crucial to maintain both the resting membrane potential and the concentration gradients of sodium and potassium across the sarcolemma ($[Na^+]_{out}$ = 145 mM,

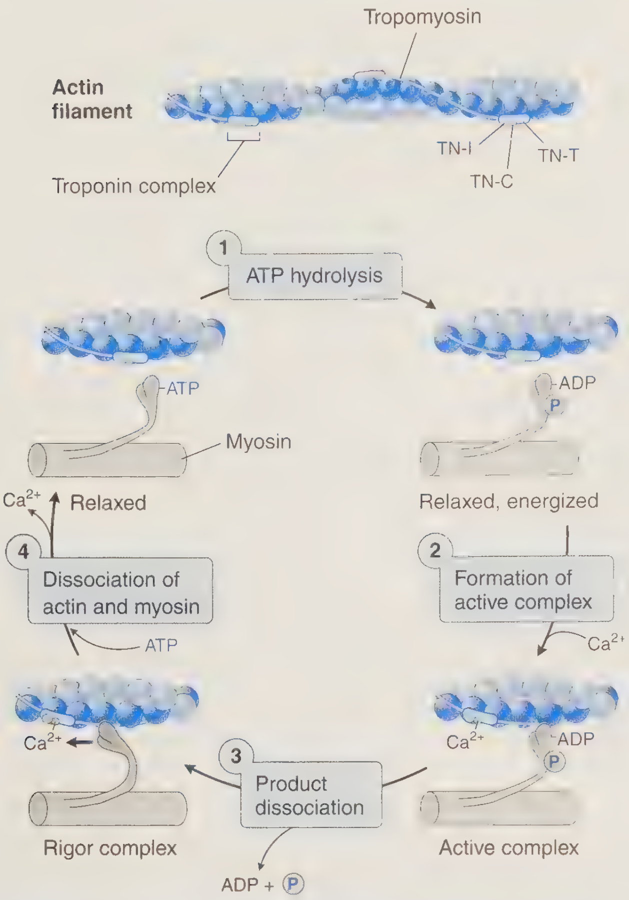


**Figure 18-2. Cardiac Contractile Proteins and the Contraction Cycle.** During contraction, myosin ratchets along actin filaments, resulting in an overall shorter sarcomere length. Actin filaments (top) consist of two actin proteins wound around one another, three troponin proteins (TN-I, TN-C, and TN-T), and tropomyosin. In the absence of $Ca^{2+}$, tropomyosin is oriented on actin so that it inhibits the interaction of actin with myosin. The contraction cycle is shown in the bottom panel and occurs in a four-step process. 1. Cardiac myocyte contraction begins with hydrolysis of ATP to ADP by myosin; this reaction energizes the myosin head. 2. $Ca^{2+}$ released from the sarcoplasmic reticulum binds to TN-C; this reaction causes a conformational change in tropomyosin that allows myosin to form an active complex with actin. 3. Dissociation of ADP from myosin allows the myosin head to bend; this bending pulls the Z lines closer together, and, thus, shortens the I band (not shown). This contracted state is often referred to as a rigor complex because muscle will stay in a contracted state unless there is sufficient ATP available to displace the myosin heads from actin. 4. Binding of a new ATP molecule to myosin allows the actin–myosin complex to dissociate. $Ca^{2+}$ also dissociates from TN-C, and the contraction cycle is repeated.

$[Na^+]_{in} = 15$ mM, $[K^+]_{out} = 5$ mM, $[K^+]_{in} = 150$ mM). Sodium pump activity is closely linked to the intracellular calcium concentration via the sodium–calcium exchanger; this antiporter exchanges sodium and calcium in both directions across the sarcolemma. Changes in the concentration of either sodium or calcium ions inside or outside the cell affect the direction and magnitude of sodium–calcium exchange. Under normal conditions, the low intracellular sodium concentration favors sodium influx and calcium efflux. Some drugs take advantage of the functional coupling between the sodium pump and the sodium–calcium exchanger to exert their effect as positive inotropes. Digoxin, discussed in the introductory case and described in detail below, is the prototype of an inotropic agent that acts by inhibiting the sodium pump. Finally, a sarcolemmal calcium pump helps to maintain calcium homeostasis by actively extruding calcium from the cytoplasm after cardiac contraction. High concentrations of ATP, thus, favor calcium removal (relaxation), both directly via the calcium pump and indirectly via the sodium pump.

## Calcium Storage and Release

In the SR, the calcium release channel (**ryanodine receptor**) and the calcium pump (**SERCA**) are critical to the regulation of contractility (Fig. 18-3). Proper contraction requires both that $Ca^{2+}$ release is adequate to stimulate contraction and that $Ca^{2+}$ reuptake is sufficient to permit relaxation and to replenish calcium stores. Cytoplasmic concentrations of both calcium and ATP regulate the activity of both the SR calcium release channel and the SR calcium pump.

As noted above, trigger calcium opens the SR calcium release channel. Cytoplasmic calcium concentration is directly related to the number of release channels that open. A safety mechanism also exists whereby high calcium levels lead to calcium–calmodulin complex formation: These complexes inhibit calcium release by decreasing the open time of the calcium release channel. High concentrations of ATP favor the open channel conformation and, thereby, facilitate SR calcium release.

In addition to opening the SR calcium release channel, cytoplasmic calcium also stimulates the SR calcium pump. This pump provides another control mechanism to prevent a positive feedback cycle that could irreversibly deplete the SR of calcium. As calcium pumps refill the SR, the rate of $Ca^{2+}$ reuptake slows because of the declining cytoplasmic calcium concentration. ATP also favors calcium pump activity; conversely, decreased ATP impairs calcium reuptake. The latter mechanism causes the rate and extent of diastolic relaxation to decrease in ischemic myocardium.

A third mediator of calcium pump activity is **phospholamban,** a SR membrane protein that inhibits the calcium pump. High levels of intracellular **cAMP** stimulate **protein kinase A** to phosphorylate phospholamban, which removes its inhibition of the calcium pump (Fig. 18-3). Phospholamban thus controls the rate of relaxation by regulating calcium reuptake into the SR: Unphosphorylated phospholamban slows relaxation, while phosphorylated phopholamban accelerates relaxation.

## Sensitivity of Contractile Proteins to Calcium

As mentioned above, the tension developed by cardiac myocytes during contraction is directly related to the precontraction length of the sarcomere units. Increased stretch of the sarcomeres exposes more calcium binding sites on troponin C, making more sites available for actin–myosin cross bridge formation and, thereby, increasing the *sensitivity* of contractile proteins to calcium. Several other mechanisms also regulate contractile protein sensitivity. Phosphorylation of troponin I by protein kinase A (a process that, like phospholamban phosphorylation, depends on cAMP levels) decreases contractile protein sensitivity to calcium. Expression of various

**TABLE 18-1** Functional Anatomy of Cardiac Myocyte Contraction

| | |
|---|---|
| **Sarcolemma** | |
| T tubules | Invaginations of sarcolemma, facilitate ion flux across the cell membrane |
| Voltage-gated L-type calcium channels | Admit influx of trigger calcium ions when sarcolemma is depolarized |
| **Sarcoplasmic Reticulum (SR)** | |
| $Ca^{2+}$ release channels | Stimulated by trigger calcium, release internal calcium stores |
| $Ca^{2+}$-ATPase pumps | Sequester intracellular calcium in SR to end contraction |
| Terminal cisternae | Sacs at distal branches of SR that store calcium |
| **Myofibril** | |
| Sarcomere | Basic contractile unit of the myofibril |
| Myosin | Thick filament, hydrolyzes ATP for energy |
| Actin | Thin filament, provides scaffolding for myosin binding |
| Tropomyosin | Coils around actin, preventing actin-myosin binding at rest |
| Troponin complex: | Complex of three proteins that regulate actin–myosin binding: |
| Troponin T | Holds troponin complex to tropomyosin |
| Troponin I | Inhibits actin–myosin binding at rest |
| Troponin C | Binds calcium, displacing troponin I from actin–myosin binding site |

isoforms of the contractile proteins, particularly troponin T, has also been linked to altered calcium sensitivity.

### cAMP

Neurohumoral activation provides yet another mechanism to enhance contractility (Fig. 18-4). The cardiostimulatory actions of the sympathetic nervous system are mediated by activation of several adrenergic receptor subtypes located in the heart and the peripheral vasculature. Stimulation of these **G protein-coupled receptors** induces conformational changes that activate **adenylyl cyclase** activity and, thereby, elevate intracellular cAMP levels. Higher levels of cAMP activate protein kinase A, which phosphorylates multiple targets in the cell. The principal adrenergic receptor in the heart is the $\beta_1$-adrenergic receptor. $\beta_1$-Receptor stimulation increases contractility (positive **inotropy**); increases SA nodal automaticity and AV nodal conduction velocity, thus speeding heart rate (positive **chronotropy**); and facilitates diastolic relaxation (positive **lusitropy**). As shown in Table 18-3, these effects result from increased phosphorylation of a number of proteins and from increased ATP availability throughout the cell.

## PATHOPHYSIOLOGY

Many disease processes can lead to myocyte dysfunction or death, replacement of myocardial tissue by fibrous infiltration, and impaired contractility. The most common etiology of contractile dysfunction in the United States is coronary artery disease (CAD) with previous myocardial infarction; other common etiologies include systemic hypertension and

**TABLE 18-2** Regulation of Cardiac Contractility

| | |
|---|---|
| Sarcolemma | $Na^+/K^+$ ATPase (extrudes sodium from cytoplasm)<br>$Na^+/Ca^{2+}$ exchanger (bidirectional, concentration-dependent)<br>$Ca^{2+}$ ATPase (extrudes calcium from cytoplasm) |
| Sarcoplasmic reticulum | $Ca^{2+}$ release channels (inhibited by $Ca^{2+}$–calmodulin complexes)<br>$Ca^{2+}$ ATPase (inhibited by phospholamban) |
| Contractile proteins | ↑ Mechanical stretch → ↑ contractile protein sensitivity<br>phosphorylation → ↓ contractile protein sensitivity |
| Intracellular cAMP | Exerts control over numerous regulatory proteins; see Table 18-3 |

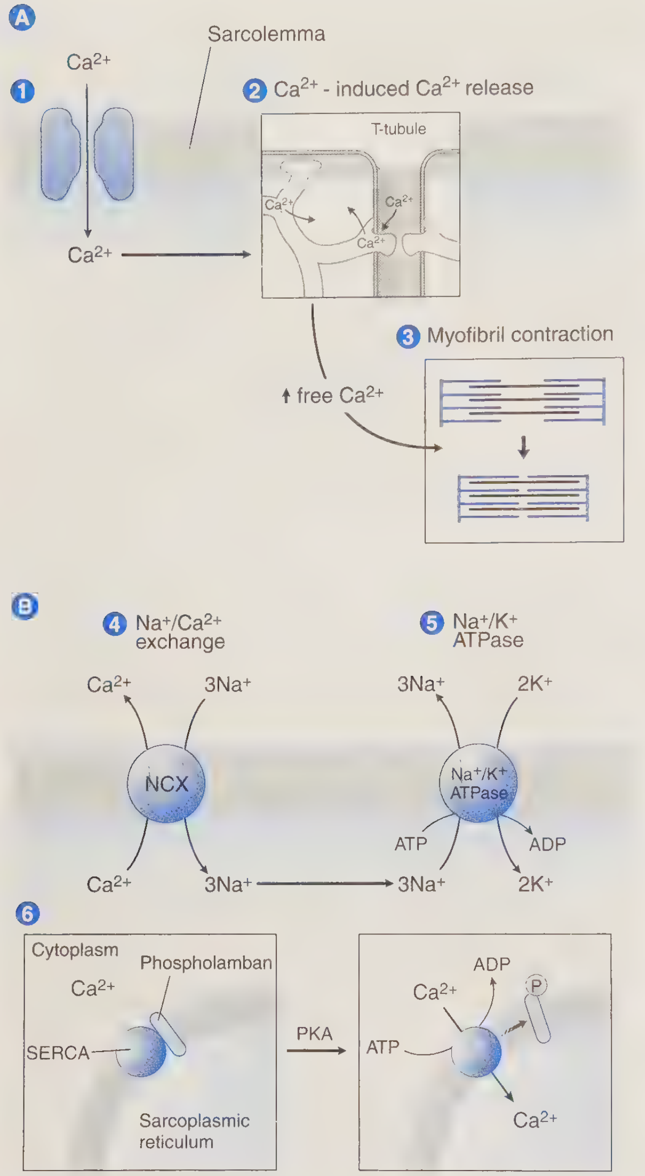

A
Sarcolemma
1
$Ca^{2+}$
$Ca^{2+}$
2 $Ca^{2+}$ - induced $Ca^{2+}$ release
T-tubule
$Ca^{2+}$
$Ca^{2+}$
$Ca^{2+}$
3 Myofibril contraction
↑ free $Ca^{2+}$
B
4 $Na^+/Ca^{2+}$ exchange
5 $Na^+/K^+$ ATPase
$Ca^{2+}$
3Na+
3Na+
2K+
NCX
Na+/K+ ATPase
ATP
ADP
$Ca^{2+}$
3Na+
3Na+
2K+
6
Cytoplasm
Phospholamban
$Ca^{2+}$
SERCA
Sarcoplasmic reticulum
PKA
ADP
$Ca^{2+}$
P
ATP
$Ca^{2+}$

**Figure 18-3. Regulation of Cardiac Myocyte $Ca^{2+}$ Flux.** **A.** During contraction: 1. Extracellular $Ca^{2+}$ enters the cardiac myocyte through $Ca^{2+}$ channels in the sarcolemma. 2. This trigger $Ca^{2+}$ induces release of $Ca^{2+}$ from the sarcoplasmic reticulum into the cytosol (so-called $Ca^{2+}$-induced $Ca^{2+}$ release). 3. The increased cytosolic $Ca^{2+}$ facilitates myofibril contraction. **B.** During relaxation: 4. The $Na^+/Ca^{2+}$ exchanger (NCX) extrudes $Ca^{2+}$ from the cytosol, using the $Na^+$ gradient as a driving force. 5. The $Na^+/K^+$ ATPase maintains the $Na^+$ gradient, thus keeping the cardiac myocyte hyperpolarized. 6. The sarcoendoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) in the sarcoplasmic reticulum membrane is tonically inhibited by phospholamban. Phosphorylation of phospholamban by protein kinase A (PKA) disinhibits the $Ca^{2-}$ ATPase, allowing sequestration of cytosolic $Ca^{2+}$ in the sarcoplasmic reticulum.

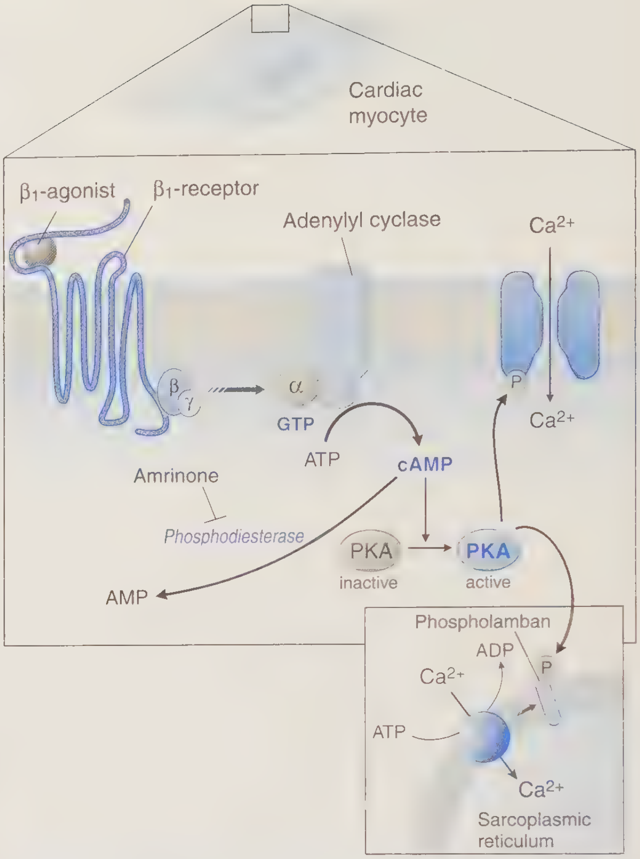

Cardiac myocyte
$\beta_1$-agonist
$\beta_1$-receptor
Adenylyl cyclase
$Ca^{2+}$
β
γ
α
GTP
ATP
cAMP
P
$Ca^{2+}$
Amrinone
Phosphodiesterase
PKA inactive
PKA active
AMP
Phospholamban
ADP
$Ca^{2+}$
P
ATP
$Ca^{2+}$
Sarcoplasmic reticulum

**Figure 18-4. Regulation of Cardiac Contractility by β-Adrenergic Receptors.** β-Adrenergic receptors increase cardiac myocyte contractility but also enhance relaxation. Binding of an agonist to $\beta_1$-adrenergic receptors on the surface of cardiac myocytes causes Gα proteins to activate adenylyl cyclase, which in turn catalyzes the conversion of ATP to cAMP. cAMP activates multiple protein kinases, including protein kinase A (PKA). PKA phosphorylates and activates sarcolemmal $Ca^{2+}$ channels and, thereby, increases cardiac myocyte contractility. PKA also phosphorylates phospholamban. The SERCA pump becomes disinhibited and pumps $Ca^{2+}$ into the sarcoplasmic reticulum; the increased rate of $Ca^{2+}$ sequestration enhances cardiac myocyte relaxation. cAMP is converted to AMP by phosphodiesterase, resulting in termination of $\beta_1$-adrenergic receptor-mediated actions. The phosphodiesterase is inhibited by amrinone, a drug that can be used in the treatment of heart failure.

valvular heart disease. A less common cause of left ventricular (LV) dysfunction is cardiomyopathy, a primary disease of the cardiac myocyte.

Progressive contractile dysfunction of the myocardium leads inevitably to the syndrome of congestive heart failure (CHF). It is important to note, however, that CHF can occur in the absence of contractile dysfunction. For example, several common cardiovascular disease states—such as acute myocardial ischemia and restrictive cardiomyopathy—are associated with abnormalities of LV relaxation and/or filling, leading to decreased chamber compliance and elevated LV diastolic pressure. This abnormal elevation of intraventricular pressure can occur even in the presence of normal systolic function, and can lead to a type of CHF known as **diastolic heart failure.** The organ-level pathology and treatment of CHF are discussed in detail in Chapter 23. Here, we focus on the salient cellular and molecular aspects of normal and abnormal contractile function.

The clinical expression of CHF often reflects the impact of neurohumoral systems that are activated by inadequate forward cardiac output. In the advanced stages of the disease, it may be difficult to determine whether the cellular abnor-

**TABLE 18-3** Effects of Increased Intracellular cAMP in Cardiac Cells

| | |
|---|---|
| Sarcolemma | ↑ Phosphorylation of voltage-gated $Ca^{2+}$ channel → ↑ contractility, heart rate, and AV conduction<br>↑ Phosphorylation of $Na^+$ pump → ↑ $Ca^{2+}$ influx into cytoplasm via $Na^+/Ca^{2+}$ exchange |
| Sarcoplasmic reticulum | ↑ Phosphorylation of phospholamban → ↑ $Ca^{2+}$ uptake and release |
| Contractile proteins | ↑ Phosphorylation of troponin I → ↓ $Ca^{2+}$ binding |
| Energy production | ↑ Glycogenolysis → ↑ ATP availability |

malities observed in the failing cardiac myocytes reflect primary cellular defects or secondary responses to extracardiac stimuli (such as circulating cytokines and neuroendocrine peptides). Nonetheless, the cellular and molecular alterations in the failing myocardium can be contrasted with the events of normal contraction in an effort to obtain mechanistic insight, and many of these changes are active areas of investigation. Study of these alterations also promises to identify potential new molecular targets for pharmacologic intervention.

## CELLULAR PATHOPHYSIOLOGY OF CONTRACTILE DYSFUNCTION

At the cellular level, changes associated with decreased cardiac contractility include dysregulation of calcium homeostasis, changes in the regulation and expression pattern of contractile proteins, and alterations in β-adrenoceptor signal transduction pathways (Fig. 18-5 and Table 18-4). As noted above, some of these alterations may result from local myocardial pathology, whereas others likely represent responses to circulating hormonal and inflammatory signals.

Altered calcium homeostasis results in prolongation of the action potential and the $Ca^{2+}$ transient associated with each contraction in failing cardiac myoctes. Mechanisms that increase the cytosolic concentration of $Ca^{2+}$ include reduced SR $Ca^{2+}$ reuptake and an increased number of sodium–calcium exchangers in the sarcolemma. As described above, efficient sequestration of calcium by the SR is essential for termination of the contraction cycle. Thus, the inability of the myocyte to regulate intracellular calcium fundamentally impairs both contractile function and diastolic relaxation.

Dysfunctional contractile proteins are produced by changes in the transcription of various genes in failing cardiac myocytes. The available data suggest that the myocytes enter a maladaptive growth phase, reverting to production of the fetal isoforms of some proteins. For example, failing myocytes increase expression of the fetal isoform of troponin T, which is potentially a more efficient contractile protein. Other contractile protein alterations described in heart failure include a reduction in phosphorylation of troponin and diminished ATP hydrolysis by myosin; both of these changes result in a slower rate of cross bridge cycling. In addition, activation of collagenase and matrix metalloproteinases may disrupt the stromal framework that maintains the structural and functional integrity of the myocardium.

Desensitization of the β-adrenergic receptor–G protein–adenylyl cyclase signaling pathway is the third major abnormal finding in cardiac myocytes of patients with congestive heart failure. Failing myocytes down-regulate the number of β-adrenergic receptors expressed at the cell surface, possibly as an adaptive response to the presence of increased neurohormonal stimulation. Sympathetic stimulation of the remaining receptors results in a smaller increase in cAMP than would occur in the presence of a normal number of receptors. This reduction in β-adrenergic sensitivity may reflect increased expression of both **β-adrenergic receptor kinase** (which phosphorylates and, thereby, inhibits β-adrenoceptors) and the **inhibitory G protein** ($G\alpha_i$). Another contributor to the reduction of β-adrenergic sensitivity may be inducible nitric oxide synthase, the expression of which is increased in CHF. The diminished response of the failing myocytes to adrenergic stimulation causes decreased phosphorylation of phospholamban, which impairs SR $Ca^{2+}$ uptake capacity. Finally, decreased cAMP levels result in a decreased ability to produce and use ATP. Together, the impaired calcium regulation and decreased cAMP levels attenuate many of the steps of cardiac myocyte contraction.

## PHARMACOLOGIC CLASSES AND AGENTS

The central roles of intracellular calcium and cAMP in cardiac myocyte contraction provide a basis for the classification of inotropic agents. The **cardiac glycosides** raise intracellular $Ca^{2+}$ concentration via inhibition of the sarcolemmal $Na^+/K^+$-ATPase (sodium pump), while **β-agonists** and **phosphodiesterase inhibitors** increase intracellular levels of cAMP. Because cAMP levels regulate the availability of calcium to the contractile proteins, the inotropic action of all of these agents results largely from their effects on intracellular calcium handling.

## CARDIAC GLYCOSIDES

The cardiac glycosides include the digitalis derivatives digoxin and digitoxin and nondigitalis agents such as ouabain. Glycosides are defined by a common chemical scaffold that includes a steroid nucleus, an unsaturated lactone ring, and one or more sugar residues. This common structural substrate suggests a common mechanism of action for these agents. In clinical practice, digoxin is both the most frequently used cardiac glycoside and the most widely used inotropic agent.

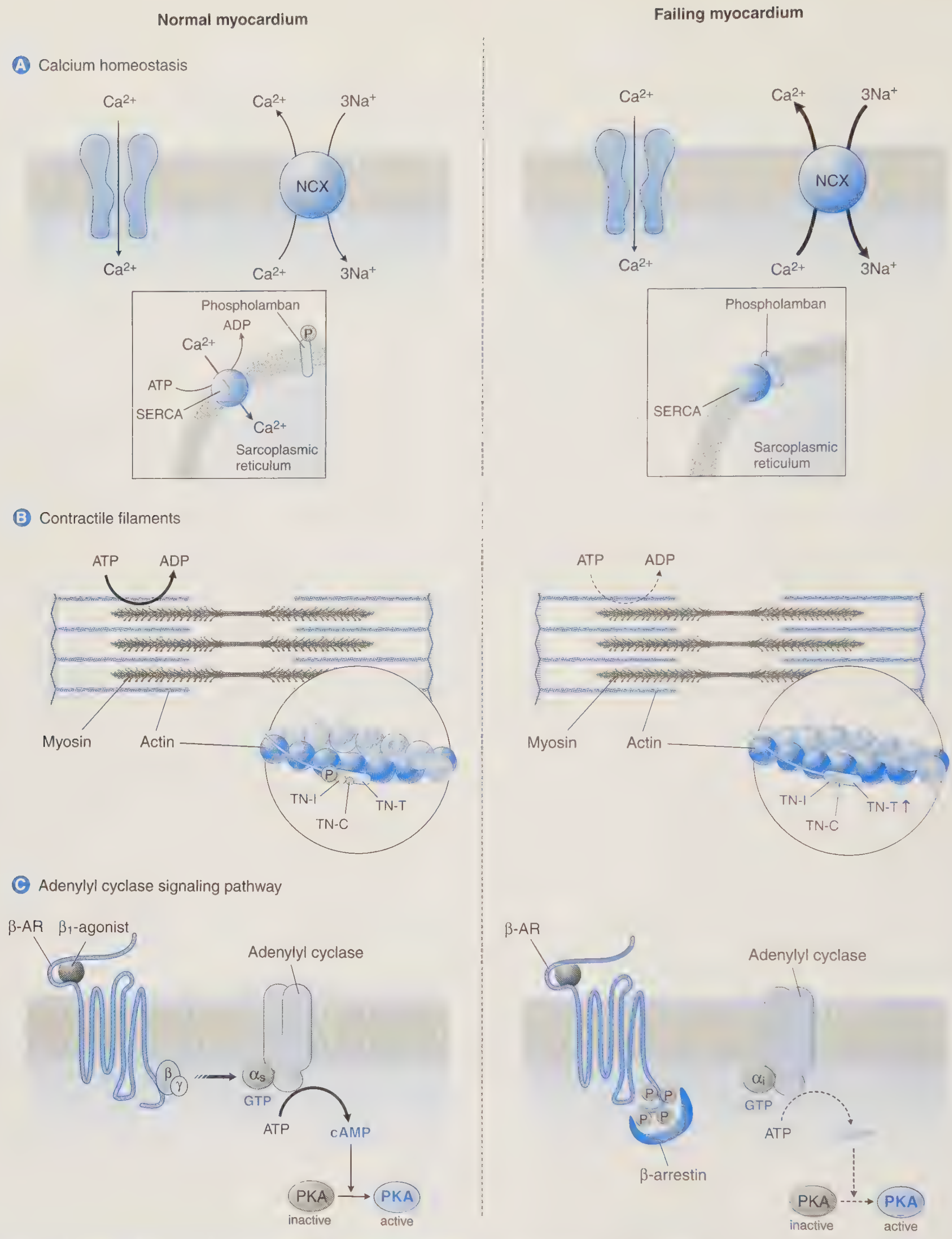

Normal myocardium
Failing myocardium
A Calcium homeostasis
Ca2+
Ca2+
3Na+
NCX
Ca2+
Ca2+
3Na+
Phospholamban
ADP
P
Ca2+
ATP
SERCA
Ca2+
Sarcoplasmic reticulum
Ca2+
Ca2+
3Na+
NCX
Ca2+
Ca2+
3Na+
Phospholamban
SERCA
Sarcoplasmic reticulum
B Contractile filaments
ATP
ADP
Myosin
Actin
P
TN-I
TN-T
TN-C
ATP
ADP
Myosin
Actin
TN-I
TN-T ↑
TN-C
C Adenylyl cyclase signaling pathway
β-AR
β1-agonist
Adenylyl cyclase
β
γ
αs
GTP
ATP
cAMP
PKA
inactive
PKA
active
β-AR
Adenylyl cyclase
P
P
P
P
β-arrestin
αi
GTP
ATP
PKA
inactive
PKA
active

**Figure 18-5. Cellular Mechanisms of Contractile Pathophysiology.** In the failing myocardium, there are derangements in $Ca^{2+}$ homeostasis, the contractile elements, and the adenylyl cyclase signaling pathway. In each panel (A, B, and C), normal myocardium is shown on the left, failing myocardium on the right. **A.** In the normal myocardium, $Ca^{2+}$ homeostasis is tightly controlled by $Ca^{2+}$ channels, including the $Na^+/Ca^{2+}$ exchanger (NCX) and the $Ca^{2+}$ ATPase (SERCA). Operation of these pathways allows the myocardium to relax during diastole. In the failing myocardium, diastolic $Ca^{2+}$ remains elevated, because phospholamban is not phosphorylated and, therefore, tonically inhibits SERCA. Also, the expression of NCX increases (large arrows), so that cytosolic $Ca^{2+}$ is extruded from the cardiac myocyte rather than being stored in the sarcoplasmic reticulum. **B.** In the normal myocardium, phosphorylation of troponin-I (TN-I) exposes the actin–myosin interaction site, and myosin effectively hydrolyzes ATP during each contraction cycle. In the failing myocardium, there is decreased phosphorylation of TN-I, resulting in less efficient actin–myosin crosslinking. Myosin does not hydrolyze ATP as efficiently (dashed arrow), further reducing the effectiveness of each contraction cycle. There is also increased expression of the fetal isoform of TN-T; the significance of this alteration is uncertain. **C.** In the normal myocardium, β-agonists stimulate cAMP formation and subsequent activation of protein kinase A (PKA). In the failing myocardium, β-arrestin binds to and inhibits the activity of β-adrenergic receptors, leading to decreased stimulation of adenylyl cyclase (dashed arrows). Expression of the inhibitory Gα isoform $G\alpha_i$ is also induced.

TABLE 18-4 Cellular Changes Associated with Decreased Contractility

| | |
|---|---|
| Sarcolemma | ↑ $Na^+/Ca^{2+}$ exchangers<br>↑ $Ca^{2+}$ pumps<br>↓ β-Adrenergic receptor expression |
| Sarcoplasmic reticulum | ↓ $Ca^{2+}$ reuptake<br>↑ Threshold for $Ca^{2+}$ release |
| Contractile proteins | ↓ Hydrolysis of ATP by myosin<br>↓ Phosphorylation of troponin I<br>↑ Expression of fetal isoform of troponin T |
| Intracellular cAMP homeostasis | ↑ β-Adrenergic receptor kinase (βARK) expression<br>↑ Inhibitory G protein ($G\alpha_i$) expression |

## Digoxin

**Digoxin** is a potent and selective inhibitor of the plasma membrane sodium pump (Fig. 18-6). Cardiac myocytes treated with digoxin extrude less sodium, and the intracellular sodium concentration rises. In turn, the increase in the intracellular sodium concentration alters the equilibrium of the sodium–calcium exchanger: Calcium efflux is decreased because the gradient for sodium entry is decreased, while calcium influx is increased because sodium efflux is increased. The net result is a rise in the intracellular calcium concentration. When the digoxin-treated cell depolarizes in response to an action potential, there is more $Ca^{2+}$ available to bind troponin C, and tension development during contraction is facilitated. In addition, the SR of the digoxin-treated cell sequesters more calcium for use in subsequent contraction cycles.

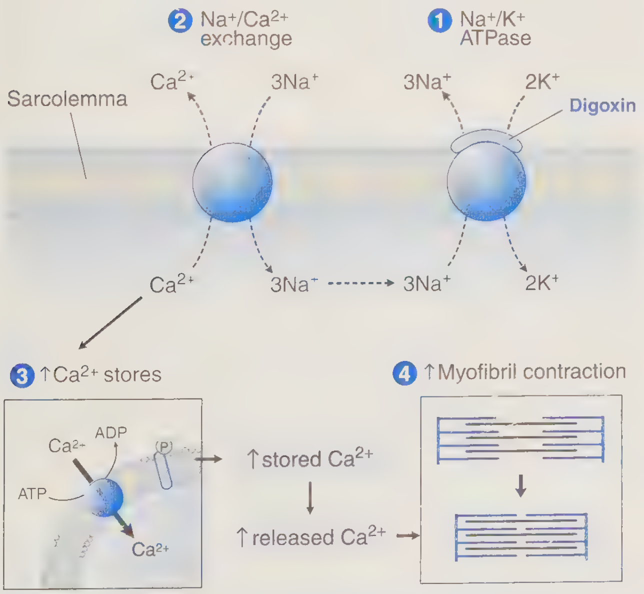


**Figure 18-6. Positive Inotropic Mechanism of Digoxin.** 1. Digoxin selectively binds to and inhibits the $Na^+/K^+$ ATPase. Decreased $Na^+$ extrusion (dashed arrows) leads to an increased concentration of cytosolic $Na^+$. 2. The increased intracellular $Na^+$ decreases the driving force for the $Na^+/Ca^{2+}$exchanger (dashed arrows) so that there is decreased extrusion of $Ca^{2+}$ from the cardiac myocyte into the extracellular space, leading to increased cytosolic $Ca^{2+}$. 3. An increased amount of $Ca^{2+}$ is then pumped by the SERCA $Ca^{2+}$ ATPase (large arrow) into the sarcoplasmic reticulum, creating a net increase in $Ca^{2+}$ that is available for release during subsequent contractions. 4. During each contraction, the increased $Ca^{2+}$ release from the sarcoplasmic reticulum leads to increased myofibril contraction, and therefore increased cardiac inotropy.

In addition to its effects on myocardial contractility, digoxin exerts autonomic effects through its binding to sodium pumps in the plasma membranes of neurons in the central and peripheral nervous systems. These effects include inhibition of sympathetic nervous outflow, sensitization of baroreceptors, and increased parasympathetic (vagal) tone. Digoxin also alters the electrophysiologic properties of the heart by a direct action on the cardiac conduction system. At therapeutic doses, digoxin decreases automaticity at the AV node, prolonging the effective refractory period of AV nodal tissue and slowing conduction velocity through the node. These combined vagotonic and electrophysiologic properties underlie digoxin's role in the treatment of patients with **atrial fibrillation** and rapid ventricular response rates; both the decreased automaticity of AV nodal tissue and the decreased conduction velocity through the node increase the degree of AV block and, thereby, decrease the ventricular response rate.

In contrast to its effects at the AV node, digoxin enhances automaticity of the infranodal (His–Purkinje) conduction system. These divergent effects at the AV node and in the His–Purkinje system explain why third-degree AV block with complete AV dissociation and accelerated idioventricular rhythm, sometimes referred to as "regularized atrial fibrillation," is a characteristic rhythm disturbance in patients with digoxin toxicity. In the introductory case, the toxic effects of an elevated serum digoxin level include the development of third-degree AV block: In effect, digoxin completely blocks conduction between the atria and ventricles. The physiologic "ventricular escape" rate of about 40 beats per minute results in a slow, regular heart rhythm characterized on the ECG by a wide QRS complex. In GW's case, the slow rate of ventricular escape could also reflect the negative chronotropic effect of the coadministered β-blocker.

Digoxin has a *narrow therapeutic window*, and prevention of digoxin toxicity depends on a complete understanding of the pharmacokinetics of this agent (Table 18-5). Several specific aspects of digoxin pharmacokinetics merit emphasis. *Chronic renal failure* reduces both the volume of distribution and the clearance of digoxin, obligating a reduction in both the loading dose and the maintenance dose of the drug (see Chapter 3). *Hypokalemia* increases the myocardial concen-

TABLE 18-5 Pharmacokinetics of Digoxin

| | |
|---|---|
| Oral bioavailability | ~75% |
| Onset (intravenous) | ~30 minutes |
| Peak effect (intravenous) | 1–5 hours |
| Half-life | 36 hours |
| Elimination | ~90% renal excretion, proportional to GFR |
| Volume of distribution | Large (~640 L/70 kg): binds to skeletal muscle |

tration of digoxin because reductions in extracellular $K^+$ result in increased phosphorylation of the sodium pump, and digoxin has a higher binding affinity for the phosphorylated form of the pump than for the dephosphorylated form. (Conversely, increasing plasma $K^+$ can help to relieve digoxin toxicity by promoting dephosphorylation of the sodium pump.)

Digoxin also *interacts* with many drugs. These interactions can be divided into pharmacodynamic and pharmacokinetic effects. Pharmacodynamic interactions include those with β-adrenergic blockers, $Ca^{2+}$ channel blockers, and $K^+$-wasting diuretics. β-Adrenergic blockers decrease AV nodal conduction, and the combined use of β-blockers and digoxin can increase the risk of developing complete heart block. $Ca^{2+}$ channel blockers decrease cardiac contractility and can, therefore, oppose the effects of digoxin. $K^+$-wasting diuretics (such as furosemide) can decrease plasma potassium concentration, which can increase the affinity of digoxin for the $Na^+/K^+$ ATPase (see above).

Pharmacokinetic interactions can result from changes in the renal clearance, volume of distribution, or absorption of digoxin. Coadministration of digoxin with verapamil (a calcium channel blocker), quinidine (a class IA antiarrhythmic), or amiodarone (a class III antiarrhythmic) can increase digoxin levels, because of the ability of these drugs to decrease the renal clearance and/or volume of distribution of digoxin. Many antibiotics, such as erythromycin, can increase digoxin absorption by killing the enteric bacteria that would ordinarily metabolize a significant fraction of orally administered digoxin before its absorption.

In the introductory case, multiple factors contribute to the marked increase in GW's serum digoxin level. The patient experiences significant weight loss, resulting in a decrease in muscle mass and a consequent reduction in the volume of distribution of digoxin. GW's glomerular filtration rate is reduced (indicated by the elevated creatinine and blood urea nitrogen [BUN]), resulting in decreased digoxin clearance. Coadministration of an ACE inhibitor and a loop diuretic, together with a reduction in oral intake, likely also contributes to the reduction in GFR. Finally, the antiarrhythmic drug amiodarone decreases both the renal clearance and volume of distribution of digoxin. Together, these factors elevate the serum digoxin concentration to 5.8 ng/mL, a level that is nearly six times the typical therapeutic concentration. To put this value into perspective, toxic effects, such as ventricular ectopy, begin to appear at digoxin concentrations of 2–3 ng/mL.

Treatment of digoxin toxicity relies on normalizing plasma $K^+$ levels and minimizing the potential for ventricular arrhythmias, usually by treating with lidocaine. In addition, life-threatening digoxin toxicity can be treated with **antidigoxin antibodies.** These polyclonal antibodies form 1:1 complexes with digoxin that are rapidly cleared from the body. Fab fragments of these antibodies (i.e., the portion of the antibody that interacts with antigen) have been shown to be less immunogenic than antidigoxin IgG, and to have a larger volume of distribution, more rapid onset of action, and increased clearance compared to the intact IgG.

It may seem counterintuitive to administer digoxin (a positive inotrope) and the β-blocker carvedilol (a negative inotrope) simultaneously to the patient in the introductory case. Both agents have been shown to provide benefit in patients with CHF, however. In fact, β-blockers have recently been shown to reduce mortality by 30% or more in CHF patients. The mechanism by which β-blockers reduce mortality remains unclear; it has been postulated that these receptor antagonists counteract the cardiotoxic effects of the chronic sympathetic stimulation induced in CHF. In the case of GW, the benefit of digoxin is most likely related to its positive effect on contractile function, while the benefit of the β-blocker may reflect long-term changes in cellular morphology and myocardial remodeling. This issue is discussed in greater detail in Chapter 23.

Several large randomized trials provide a consistent picture of the clinical efficacy and limitations of digoxin. These trials indicate that withdrawal of digoxin in heart failure patients leads to a deterioration in clinical status compared to patients who continue digoxin therapy. Withdrawal is also associated with deterioration in exercise capacity and increased frequency of hospitalization for worsening heart failure. The use of digoxin does not have a significant impact on survival for patients with CHF, however. In short, while digoxin has not been shown to improve survival, it does palliate symptoms, improve functional status, and reduce hospitalization rates; these clinical benefits may provide significant improvement in the quality of life for patients with CHF.

## Digitoxin

**Digitoxin** is a less frequently used digitalis preparation that may be preferable to digoxin in selected clinical circumstances. Digitoxin is structurally identical to digoxin except for the presence (digoxin) or absence (digitoxin) of a hydroxyl group at position 12 of the steroid nucleus. This structural modification renders digitoxin less hydrophilic than digoxin, and significantly alters the pharmacokinetics of the drug. In particular, digitoxin is metabolized and excreted primarily by the liver; the fact that its clearance does not depend on renal excretion makes digitoxin a suitable alternative to digoxin for the treatment of patients with renal insufficiency. However, digitoxin has a very long half-life (approximately 7 days) compared even to the long half-life of digoxin (about

36 hours). Therefore, the management of digitoxin toxicity is more complex than that of digoxin toxicity.

## β-ADRENERGIC RECEPTOR AGONISTS

$\beta_1$-Adrenoceptor stimulation supports cardiac performance by increasing the fractional shortening of cardiac muscle with each contraction. This positive inotropic effect results in a greater stroke volume for any given end-diastolic volume. β-Agonists also have a positive chronotropic effect, increasing heart rate in a relatively linear dose-dependent manner. The net effect of these inotropic and chronotropic effects is to increase cardiac output.

$$\text{Cardiac Output (CO)} = \text{Heart Rate (HR)} \times \text{Stroke Volume (SV)} \qquad \text{Equation 18-1}$$

A third, but less widely appreciated, mechanism by which β-agonists support cardiac performance is by enhancing the rate and extent of diastolic relaxation. This is a critical permissive effect of $\beta_1$-receptor stimulation, because it facilitates maintenance of adequate left ventricular filling (i.e., preservation of left ventricular end-diastolic volume) despite the reduction in diastolic filling time that occurs as heart rate increases.

In the peripheral circulation, the effects of sympathetic stimulation are more complex. Activation of peripheral $\beta_2$-receptors dilates vascular smooth muscle, but $\alpha_1$-receptor stimulation constricts vascular smooth muscle. Thus, *$\beta_2$-receptor stimulation typically decreases systemic vascular resistance (SVR) and afterload, while $\alpha_1$-receptor stimulation increases these parameters.* Dopamine receptors in the splanchnic and renal circulations also modulate the resistance vessels in these vascular beds. In general, these combined peripheral effects determine the overall hemodynamic effect of a given sympathomimetic agent. For example, a nonspecific β-agonist (i.e., an agent that stimulates both $\beta_1$ and $\beta_2$ receptors) could relieve CHF symptoms such as pulmonary congestion in one patient (because of the net effects of increased contractility and peripheral vasodilation/reduced afterload), while a mixed $\beta_1$ (increased contractility) and $\alpha_1$ (increased SVR) agonist could have a neutral or even negative impact on global cardiac performance. It merits emphasis that *the differential activation of receptor subtypes is influenced both by the agent selected and the dose at which the selected agent is administered.* For example, dopamine administered at low infusion rates (2–5 μg/kg/min) has an overall cardiostimulatory effect (caused by increased contractility and decreased SVR), while the same drug infused at higher rates (>10 μg/kg/min) has an overall impact that is largely related to $\alpha_1$-receptor activation. Thus, the pharmacodynamic effects of the agent (see Table 18-6) must be considered in the context of the patient's overall hemodynamic profile; this often requires placement of hemodynamic monitoring catheters to quantify intracardiac filling pressures, systemic vascular resistance, and cardiac output.

Clinical use of the sympathomimetic inotropes is generally reserved for short-term support of the failing circulation. This is attributable to both the side effect profile of these agents and their pharmacodynamic properties. In general, sympathomimetic agents that stimulate myocardial β-adrenergic receptors share the side effect profile of tachycardia, arrhythmia, and increased myocardial oxygen consumption. These agents also induce tolerance rapidly via down-regulation of adrenergic receptors at the cell surface of target organs. In addition, the sympathomimetic amines have low oral bioavailability, and are typically administered by continuous intravenous infusion.

### Dopamine

**Dopamine** (DA) is an endogenous sympathomimetic amine that functions as a neurotransmitter; it is also a biosynthetic precursor of norepinephrine and epinephrine (see Chapter 8, Adrenergic Pharmacology). Like norepinephrine, DA activates cardiac $\beta_1$-adrenergic receptors, thereby increasing contractility and heart rate. Unlike norepinephrine, at low doses, dopamine has a vasodilator effect in the periphery by stimulating dopaminergic D1 receptors in the renal and mesenteric vascular beds. This regional vascular dilation reduces the impedance to left ventricular ejection (afterload). At intermediate doses, DA causes more widespread vasodilation and a greater reduction in systemic vascular resistance, although the preferential dilation of renal and mesenteric beds is lost at this midrange dose. At higher doses, activation of $\alpha_1$-receptors predominates, leading to generalized vasoconstriction and an increase in afterload.

Despite this complex pharmacology, DA finds wide clinical application in patients with distributive shock (e.g., sepsis

**TABLE 18-6** Receptor Selectivity of Sympathomimetics

| | Receptor Type | | | | |
|---|---|---|---|---|---|
| | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ | D1 |
| **Agent** | Vasoconstricts peripheral vessels | Presynaptic inhibition at NE synapse | Increases heart rate, contractility, diastolic relaxation | Vasodilates peripheral vessels | Low doses vasodilate renal vessels |
| Dopamine | + | | ++ | ++ | ++ |
| Dobutamine | +/– | | ++ | + | |
| Epinephrine | ++ | ++ | ++ | ++ | |
| Norepinephrine | ++ | ++ | ++ | | |

Also similar to the β-agonists, PDE inhibitors have found clinical utility in short-term support of the severely failing circulation. Widespread application of oral *amrinone* has been limited by the development of clinically significant thrombocytopenia in about 10% of patients. Clinical trials of *milrinone* (an analogue of amrinone) have demonstrated a statistically significant *increase* in mortality in heart failure patients; the increase exceeds 50% in patients with the most severe symptoms (NYHA Class IV: symptoms at rest). Although the early data from clinical trials of a third novel PDE inhibitor, **vesnarinone,** suggested that this drug could have a favorable impact on survival in patients with advanced CHF, detailed review of the complete trial data have also demonstrated an increase in mortality in the treatment group.

**Calcium sensitizing drugs,** such as **levosimendan,** are a novel class of PDE inhibitors for which only early clinical trial data are available. These calcium sensitizers, which also have the characteristic "ino-dilator" actions of the PDE inhibitors, augment myocardial contractility by enhancing the sensitivity of troponin C to calcium. This potentiating effect increases the extent of actin–myosin interactions at any given concentration of intracellular calcium; that is, without a concomitant increase in ATP requirements. The net hemodynamic impact of these promising drugs therefore includes: (1) the characteristic vasomotor effects of the PDE inhibitors; (2) the positive inotropic effects of PDE inhibition; and (3) positive effects on the energetics of myocardial contraction.

## Conclusion and Future Directions

Knowledge of the cellular and molecular basis for myocardial contraction has provided several pharmacologic strategies designed to increase myocardial contractility in patients with heart failure attributable to left ventricular systolic dysfunction. By inhibiting the sodium pump, *digoxin* raises intracellular calcium levels and, thereby, increases contractile force. This drug is the only oral inotropic agent in wide clinical use today. Although digoxin has no demonstrable impact on the mortality of patients with heart failure, it helps alleviate symptoms and improves functional capacity. Digoxin also slows AV nodal conduction, an effect that is very useful in treating patients with atrial fibrillation and rapid ventricular response rates. The *β-agonists,* including the endogenous amines *dopamine, norepinephrine,* and *epinephrine* and the synthetic amine *dobutamine,* act through G protein-mediated elevation of intracellular cAMP to enhance both myocardial contractility and diastolic relaxation. The latter effect allows the left ventricle to fill adequately during diastole, despite the increase in heart rate that is stimulated by these agents. β-agonists are administered intravenously, and provide short-term hemodynamic support to patients with cardiogenic circulatory failure. The longer-term utility of these agents has been limited both by the lack of an oral formulation with acceptable bioavailability and by the adverse side effect profile of these drugs. *PDE inhibitors* act as positive inotropes and as mixed arterial and venous dilators by increasing the levels of cyclic AMP in the heart and vascular smooth muscle. The increased mortality associated with longer-term use of these agents has similarly restricted their role to the short-term management of severe heart failure.

New classes of pharmacologic agents for the augmentation of myocardial contractility are under active investigation. These agents are directed at a variety of biochemical targets, including the signaling systems that regulate the synthesis of contractile proteins (e.g., **cardiac neuregulins**). Alternative strategies attempt to preserve myocardial contractility by inhibiting the effects of proinflammatory cytokines associated with heart failure. For example, **endothelin receptor antagonists** attenuate the progression of LV dysfunction and increase survival in animal models of CHF. The PDE inhibitor *vesnarinone,* a positive inotrope that has been associated with increased mortality in clinical trials, is now being examined for its anticytokine potential. Finally, **gene therapy** methods to increase contractility include the delivery of genes, with cardiac-specific promoters, that alter the production of contractile proteins, channels, and regulators. At the present time, the most promising candidates for gene therapy include the SR calcium pump, phospholamban, and cardiac troponin I.

## Suggested Readings

Braunwald E, ed. Heart Disease: A Textbook of Cardiovascular Medicine. 6th Ed. Philadelphia: WB Saunders, 2001. (*Encyclopedic reference that includes a good survey of pharmacologic agents, trials, and new approaches.*)

Figgitt DP, Gillies PS, Goa KL. Levosimendan. Drugs 2001; 61: 613–627. (*Describes properties of one of the best-studied newer calcium sensitizers.*)

Hunter JJ, Chien KP. Mechanisms of disease: Signaling pathways for cardiac hypertrophy and failure. N Engl J Med 1999; 341: 1276–1283. (*Reviews recent advances in the molecular signaling of cardiac dysfunction.*)

Katz AM. Physiology of the Heart. 2nd Ed. New York: Raven Press, 1992. (*Comprehensive account of cardiac physiology. Chapters 7–14, on contractile function and regulation, explain in great detail the physiology summarized in this chapter.*)

Lilly LS, ed. Pathophysiology of Heart Disease. 2nd Ed. Baltimore: Lippincott Williams & Wilkins, 1997. (*Excellent introduction to cardiovascular medicine. Chapters 1 (Basic Cardiac Structure and Function), 9 (Heart Failure), and 17 (Cardiovascular Drugs) relate to the physiology, pathophysiology, and pharmacology of contractile function.*)

Mittmann C, Eschenhagen T, Scholz H. Cellular and molecular aspects of contractile dysfunction in heart failure. Cardiovasc Res 1998;39:267–275. (*Reviews current understanding of the cellular pathophysiology of heart failure.*)

Rathore SS, Wang Y, Krumholz HM. Sex-based differences in the effect of digoxin for the treatment of heart failure. N Engl J Med 2002;347:1403–1411. (*Post-hoc analysis of a randomized clinical trial showing increased mortality in women, but not in men, using dogoxin.*)

Webster KA, Bishopric NH. Diastolic function and dysfunction: Molecular aspects and gene therapy prospects for diastolic failure. Cardiol Clin 2000;28:621–635. (*Reviews current gene therapy approaches.*)

UNIVERSITY COLLEGE Library CORK

Drug Summary Table

## Pharmacology of Cardiac Contractility

| Drug | Clinical Uses | Serious and Common Adverse Effects | Contraindications | Therapeutic Considerations |
|---|---|---|---|---|
| **CARDIAC GLYCOSIDES**<br>*Mechanism—Increase intracellular $Ca^{2+}$ by inhibiting $Na^+/K^+$ ATPase* | | | | |
| **Digoxin**<br>**Digitoxin** | Left ventricular systolic dysfunction<br>Atrial fibrillation/flutter<br>Paroxysmal supraventricular tachycardia (PSVT) | Arrhythmias (ventricular extrasystoles, AV block)<br>GI effects (anorexia, nausea, vomiting)<br>CNS effects (headache, fatigue, blurred yellow vision) | Hypokalemia increases myocardial concentration and toxicity<br>Verapamil, quinidine, amiodarone, erythromycin, tetracycline (displacement from plasma protein binding sites) | Digoxin is the only widely used oral inotropic agent<br>Renal failure requires reduction of loading and maintenance doses of digoxin<br>Digitoxin has very long $t_{1/2}$ (170 hours)<br>Digitoxin has hepatic metabolism and biliary excretion |
| **β-ADRENERGIC AGONISTS**<br>*Mechanism—Increase cAMP by activating G protein-coupled receptors* | | | | |
| **Dopamine** | Distributive or cardiogenic shock, CHF | All β-adrenergic agonists can cause arrhythmia, hypertension/hemorrhage, CNS effects (tremor, anxiety)<br>Epinephrine can also cause pulmonary edema | | Receptor selectivity: $D1=D2>\beta>\alpha$ |
| **Dobutamine** | Cardiogenic shock | | Caution in atrial fibrillation, because increases AV conduction | Receptor selectivity: $\beta_1>\beta_2$ |
| **Epinephrine** | Anaphylactic shock, cardiac resuscitation, bronchospasm, glaucoma | | Enhanced cardiac effects in hyperthyroidism and cocaine use | Receptor selectivity: $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ |
| **Norepinephrine** | Shock | | Reduces renal blood flow | Receptor selectivity: $\alpha_1$, $\alpha_2$, $\beta_1$ |
| **PHOSPHODIESTERASE (PDE) INHIBITORS**<br>*Mechanism—Increase cAMP by inhibiting the PDE enzymes that hydrolyze it* | | | | |
| **Amrinone**<br>**Milrinone** | CHF (Short-term therapy in hospitalized patients only) | PDE inhibitors can cause ventricular arrhythmias<br>Amrinone causes thrombocytopenia in ~10% of patients | Valvular disease, myocardial infarction, atrial fibrillation/flutter | Amrinone does not increase survival<br>Milrinone increases mortality |

# 19

# Pharmacology of Volume Regulation

Mallar Bhattacharya and Seth L. Alper

**Introduction**
**Case**
**Physiology of Volume Regulation**
  Determinants of Intravascular Volume
  Volume Sensors
  Volume Signals
    *Renin-Angiotensin–Aldosterone System*
    *Natriuretic Peptides*
    *Antidiuretic Hormone*
    *Renal Sympathetic Nerves*
  Renal Control of Na⁺ Excretion
    *Proximal Tubule*
    *Thick Ascending Limb of the Loop of Henle*
    *Distal Convoluted Tubule*
    *Collecting Duct*
**Pathophysiology of Edema Formation**
  Congestive Heart Failure
  Cirrhosis
  Nephrotic Syndrome
**Pharmacologic Classes and Agents**
  Agents That Modify Volume Signals
    *Inhibitors of the Renin–Angiotensin System*
    *B-Type Natriuretic Peptide*
    *Antidiuretic Hormone (ADH) Antagonists*
  Agents That Decrease Renal Na⁺ Reabsorption
    *Carbonic Anhydrase Inhibitors*
    *Osmotic Diuresis*
    *Loop Diuretics*
    *Thiazides*
    *Collecting Duct (Potassium-Sparing) Diuretics*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Coordinate regulation of volume homeostasis and vascular tone maintain adequate tissue perfusion in response to varying environmental stimuli. This chapter discusses the pharmacologically relevant physiology of volume regulation, with emphasis on the hormonal pathways and renal mechanisms used to modulate volume. (Control of vascular tone is discussed in Chapter 20, Pharmacology of Vascular Tone.) Dysregulation of volume homeostasis can result in edema, the pathologic accumulation of fluid in the extravascular space. Pharmacologic modulation of volume is targeted at reducing volume excess; this is an effective treatment for hypertension and congestive heart failure (CHF), as well as cirrhosis and the nephrotic syndrome. The two broad classes of pharmacologic agents used to modify volume status are modulators of neurohormonal signals (e.g., angiotensin converting enzyme inhibitors) and diuretics, agents that increase renal $Na^+$ excretion. In addition, drugs that modify volume signals have numerous other clinically important effects on the body, because these volume signals act as diverse hormonal modulators in a number of physiologic pathways. Many of the clinical applications of these agents are discussed in further detail in Chapter 23, Integrative Cardiovascular Pharmacology.

## Case

70-year old Mr. Reilly, Grand Poo-Bah of the Boca Raton Cigar Club, is taken by ambulance to the emergency room at 1 AM after waking up with shortness of breath for the fourth night in a row. Each time he "felt tight in the chest" and "couldn't get a breath;" this discomfort was relieved somewhat by sitting up in bed. He also recalls previous episodes of shortness of breath while climbing stairs at the club.

Physical exam reveals tachycardia, mild hypertension, pedal edema (edema of the feet and lower legs), and bilateral pulmonary crackles on inspiration. Serum chemistries show no elevation of creatine phosphokinase but mildly elevated creatinine and blood urea nitrogen (BUN). The electrocardiogram shows evidence of an old myocardial infarction. Echocardiography reveals diminished left ventricular ejection fraction (the fraction of blood in the ventricle at the end of diastole that is ejected when the ventricle contracts) without ventricular dilatation.

Based on the clinical findings of decreased cardiac output, pulmonary congestion, and peripheral edema, Mr. Reilly is diagnosed with acute CHF. His increased creatinine and BUN also indicate an element of renal insufficiency. Pharmacologic therapy is started, including a positive inotrope, a coronary vasodilator, an antihypertensive angiotensin converting enzyme (ACE) inhibitor, and a loop diuretic. After Mr. Reilly's condition stabilizes, the dose of the loop diuretic is decreased and then discontinued. Elective coronary angiography reveals significant stenosis of the left anterior descending coronary artery. Mr. Reilly undergoes balloon angioplasty and stent placement and remains stable as an outpatient with his discharge drug regimen accompanied by a low-salt, low-fat diet.

## QUESTIONS

1. What mechanisms led to Mr. Reilly's pulmonary congestion and pedal edema?
2. Why was Mr. Reilly given a loop diuretic?
3. How do ACE inhibitors improve cardiovascular hemodynamics?
4. What other kinds of diuretics are available, and why were they not chosen in this acute setting?

## PHYSIOLOGY OF VOLUME REGULATION

An intricate set of mechanisms sense, signal, and modulate changes in plasma volume. Volume sensors are located throughout the vascular tree, including in the atria and in the kidneys. These sensors regulate blood pressure and total volume status within a narrow set-point. Many of the signals activated by these sensors are hormones, while others are neural circuits. The end result of these signaling mechanisms is to alter vascular tone and to regulate renal $Na^+$ reabsorption and excretion. Vascular tone maintains end-organ tissue perfusion; changes in renal $Na^+$ excretion alter total volume status.

## DETERMINANTS OF INTRAVASCULAR VOLUME

Intravascular volume is a small proportion of total body water, but the amount of fluid in the vascular compartment critically determines the extent of tissue perfusion. Approximately ⅔ of total body water is intracellular, while ⅓ is extracellular. Of the extracellular fluid (ECF), approximately ¾ resides in the interstitial space, while ¼ of ECF is plasma.

Fluid exchange between plasma and interstitial fluid occurs as a result of changes in capillary permeability, oncotic pressure, and hydrostatic pressure. Capillary permeability is largely determined by the junctions between individual endothelial cells lining a vascular space. The capillary beds of some organs are more permeable than others and, as a result, allow greater intercompartmental fluid shifts. In the context of inflammation and other pathologic conditions (see below), increased capillary permeability allows proteins and other osmotically active agents to shift between intravascular and perivascular compartments. Oncotic pressure is determined by the molecular solute components of a fluid space that are differentially partitioned between adjacent compartments (such constituents are said to be osmotically active). Because albumin, globulins, and other large plasma proteins are normally confined to the plasma space, these proteins act as osmotically active agents to retain water in the vascular space. The hydrostatic pressure gradient across the capillary barrier between compartments is another force for water movement between the compartments. An elevated intracapillary pressure favors increased transudation of fluid from plasma into the interstitial space.

The relationship between fluid filtration and capillary permeability, oncotic pressure, and hydrostatic pressure is represented by the following equation:

$$\text{Fluid Filtration} = K_f[(P_c - P_{if}) - (\Pi_c - \Pi_{if})] \quad \text{Equation 19-1}$$

where $K_f$ = capillary permeability, $P_c$ = capillary hydrostatic pressure, $P_{if}$ = interstitial fluid hydrostatic pressure, $\Pi_c$ = capillary oncotic pressure, and $\Pi_{if}$ = interstitial fluid oncotic pressure. This equation emphasizes that transcapillary fluid movement is governed by intercompartmental gradients, rather than by the absolute value of each compartmental pressure. Note that *the hydrostatic and oncotic gradient terms have opposing vectors* and, therefore, favor fluid movement in opposing directions. $\Delta P_c$ Normally favors transudation from the capillary lumen to the interstitium; whereas, $\Delta\Pi_c$ normally favors fluid retention within the capillary lumen.

The extent of fluid filtration that occurs along the length of the capillary differs for each tissue's capillary bed and is determined by cellular and junctional permeability properties of tissue-specific capillary endothelial cells. In the example shown in Fig. 19-1, liver capillaries filter fluid into the interstitium along their entire length. At the arterial end of the capillary bed, $(P_c + \Pi_{if})$ exceeds $(P_{if} + \Pi_c)$, thus favoring plasma filtration from the capillary into the interstitial space. $P_c$ gradually decreases along the length of the capillary, and the rate of fluid filtration into the interstitium decreases. At the venous end of the capillary, hydrostatic fluid filtration and oncotic fluid absorption are almost balanced. In capillary beds of other tissues, however, the integrated oncotic pressure gradient favoring fluid flow into the capillary balances the integrated hydrostatic pressure gradient, resulting in no net volume change between the vascular and interstitial spaces. Liver sinusoids, which transfer fluid into the interstitial space during perfusion, return this fluid to the circulation via lymphatic flow. Thus, the physiologic steady-state of extracellular fluid represents a balance of driving forces between fluids of the intravascular and interstitial compartments. Pathologic alterations in the determinants of transcapillary fluid shifts, coupled with changes in renal $Na^+$ handling, can result in the formation of edema, as discussed below.

## VOLUME SENSORS

Vascular volume sensors can be divided into low-pressure and high-pressure feedback systems. The low-pressure system consists of the atria and pulmonary vasculature. In re-

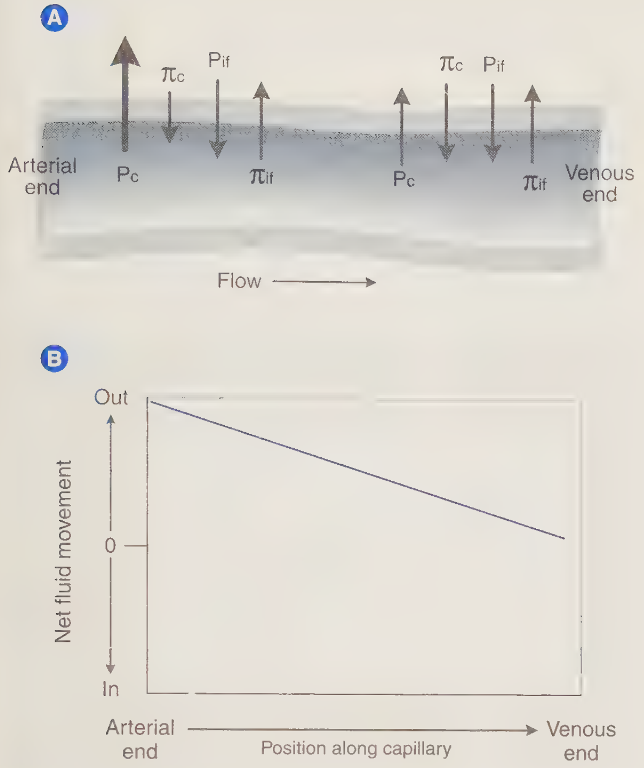


**Figure 19-1. Capillary Fluid Filtration.** The balance of hydrostatic pressure and oncotic pressure determines fluid filtration along the capillary. The example shown here is for a hypothetical capillary where fluid filtration exceeds fluid reabsorption. **A.** At the arterial end of the capillary, the capillary hydrostatic pressure ($P_c$) is large (indicated by a long arrow), and the sum of $P_c$ and interstitial oncotic pressure ($\pi_{if}$) exceeds the sum of interstitial hydrostatic pressure ($P_{if}$) and capillary oncotic pressure ($\pi_c$). Therefore, fluid moves out of the capillary into the interstitial space. As fluid continues to filter along the length of the capillary, the increased fluid filtration results in decreased $P_c$ and increased $\pi_c$, thus decreasing the driving force for fluid filtration from the capillary to the interstitium. Throughout the length of the capillary, $P_{if}$ and $\Pi_{if}$ remain relatively constant. **B.** A graphic representation of fluid movement along the capillary length shows the decreasing driving force for fluid filtration into the interstitium. In the hypothetical capillary shown here, fluid is filtered into the interstitium along the entire capillary length; lymphatic vessels eventually return the excess interstitial fluid to the systemic circulation (not shown).

sponse to decreased wall stress (e.g., caused by decreased intravascular volume), peripheral nervous system cells lining the atrial and pulmonary vasculature transmit a signal to noradrenergic neurons in the central nervous system (CNS) (medulla). This signal is then relayed to the hypothalamus and results in increased secretion of antidiuretic hormone (ADH) from the posterior pituitary gland. ADH, together with increased peripheral sympathetic tone, maintains distal tissue perfusion. In response to increased wall stress (e.g., caused by increased intravascular volume), cells of the atria also produce and secrete natriuretic peptide, promoting vasodilation and **natriuresis** (increased renal $Na^+$ excretion).

The high-pressure system consists of specialized baroreceptors in the aortic arch, carotid sinus, and juxtaglomerular apparatus. These sensors modulate hypothalamic control of ADH secretion and sympathetic outflow from the brainstem. In addition, sympathetic input stimulates the juxtaglomerular apparatus to secrete renin, a proteolytic enzyme that activates the renin-angiotensin–aldosterone system (see below).

## VOLUME SIGNALS

Together, the low-pressure and high-pressure feedback systems integrate to produce neurohumoral volume signals to reestablish volume homeostasis. The neurohormonal response to a change in volume status is controlled by at least four main systems: the renin-angiotensin–aldosterone system (RAAS), natriuretic peptides, antidiuretic hormone (ADH), and renal sympathetic nerves. The RAAS, ADH, and renal sympathetic nerves are active in situations of intravascular volume depletion, while natriuretic peptides are released in response to intravascular volume overload.

### Renin-Angiotensin–Aldosterone System

Renin is an aspartyl protease produced and secreted by the **juxtaglomerular apparatus,** a specialized set of smooth muscle cells that line the afferent and efferent arterioles of the renal glomerulus. The ultimate result of renin secretion is *vasoconstriction and $Na^+$ retention, actions that maintain tissue perfusion and increase extracellular fluid volume* (Fig. 19-2).

Three mechanisms control juxtaglomerular cell renin release (Fig. 19-3). First, a direct pressure-sensing mechanism by the afferent arteriole increases juxtaglomerular cell release of renin in response to decreased tension. The molecular mechanism of this release is unknown, but may involve prostaglandin signaling. Second, sympathetic innervation of juxtaglomerular cells promotes renin release via $\beta_1$-adrenoceptor signaling. Third, an autoregulatory mechanism known as **tubuloglomerular feedback** senses distal nephron sodium (or chloride) delivery and modulates renin release accordingly. Nephron anatomy is organized so that the distal end of the cortical thick ascending limb of each nephron is closely juxtaposed to the glomerulus of that same nephron. This spatial proximity allows regulation of glomerular activity by distal nephron electrolyte concentration and/or salt load. Macula densa cells of the cortical thick ascending limb (TAL) respond to increased lumenal NaCl delivery by generation of extracellular adenosine in the juxtoglomerular interstitium, activating a juxtaglomerular mesangial cell $A_1$ receptor. Conversely, decreased lumenal NaCl delivery activates a prostaglandin signaling cascade that seems to culminate in increased juxtaglomerular cell renin release.

Plasma renin cleaves the 14 amino acid hepatic prohormone **angiotensinogen** to the decapeptide **angiotensin I.** Angiotensin I is, in turn, cleaved to the active octapeptide **angiotensin II** (ATII) by the carboxypeptidase **angiotensin-converting enzyme** (ACE) located on the endothelial cell surface. ACE is highly expressed in the pulmonary vascular endothelium, but is also present on the surface of other endothelial cells, including those lining the coronary arteries. ACE activity regulates the local production of ATII in all vascular beds. Indeed, an incompletely understood "local"

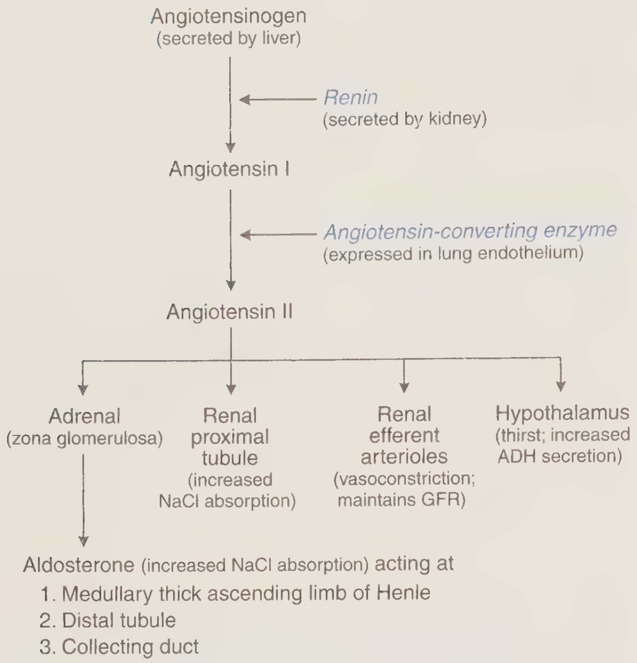

Angiotensinogen
(secreted by liver)
Renin
(secreted by kidney)
Angiotensin I
Angiotensin-converting enzyme
(expressed in lung endothelium)
Angiotensin II
Adrenal
(zona glomerulosa)
Renal
proximal
tubule
(increased
NaCl absorption)
Renal
efferent
arterioles
(vasoconstriction;
maintains GFR)
Hypothalamus
(thirst; increased
ADH secretion)
Aldosterone (increased NaCl absorption) acting at
1. Medullary thick ascending limb of Henle
2. Distal tubule
3. Collecting duct

**Figure 19-2. The Renin-Angiotensin–Aldosterone Axis.** Angiotensinogen is a prohormone secreted into the circulation by hepatocytes. Renin, an aspartyl protease secreted by juxtaglomerular cells of the kidney, cleaves angiotensinogen to angiotensin I. Angiotensin-converting enzyme, a protease expressed by pulmonary capillary endothelium (and elsewhere), then cleaves angiotensin I to angiotensin II. Angiotensin II has four actions that increase intravascular volume and maintain tissue perfusion. First, angiotensin II stimulates zona glomerulosa cells of the adrenal cortex to secrete aldosterone, a hormone that increases renal NaCl reabsorption at multiple sites along the nephron. Second, angiotensin II directly stimulates renal proximal tubule reabsorption of NaCl. Third, angiotensin II causes efferent arteriole vasoconstriction, an action that increases intraglomerular pressure and, thereby, increases the glomerular filtration rate. Fourth, angiotensin II stimulates hypothalamic thirst centers and promotes antidiuretic hormone secretion.

renin–angiotensin system also seems to be expressed in the vasculature, producing these substances as autocrine factors independently of the kidney and liver. ACE has a broad proteolytic substrate specificity that includes bradykinin and other kinins, which are venodilatory autacoids released in response to inflammation. For this reason, ACE is also known as **kininase II.** Kininase activity has important pharmacologic consequences, as discussed below.

AT II has at least four physiologic actions: (1) stimulation of aldosterone secretion by zona glomerulosa cells of the adrenal glands; (2) increased reabsorption of NaCl at the proximal tubule and at other nephron segments; (3) stimulation of thirst and ADH secretion; and (4) arteriolar vasoconstriction. All four of these actions increase intravascular volume and, therefore, help to maintain perfusion pressure: aldosterone secretion increases distal tubule $Na^+$ reabsorption; proximal tubule NaCl reabsorption increases the fraction of filtered $Na^+$ that is reabsorbed; stimulation of thirst increases the free water absorbed into the vasculature; secretion of ADH increases collecting duct free water absorption; and arteriolar vasoconstriction maintains blood pressure.

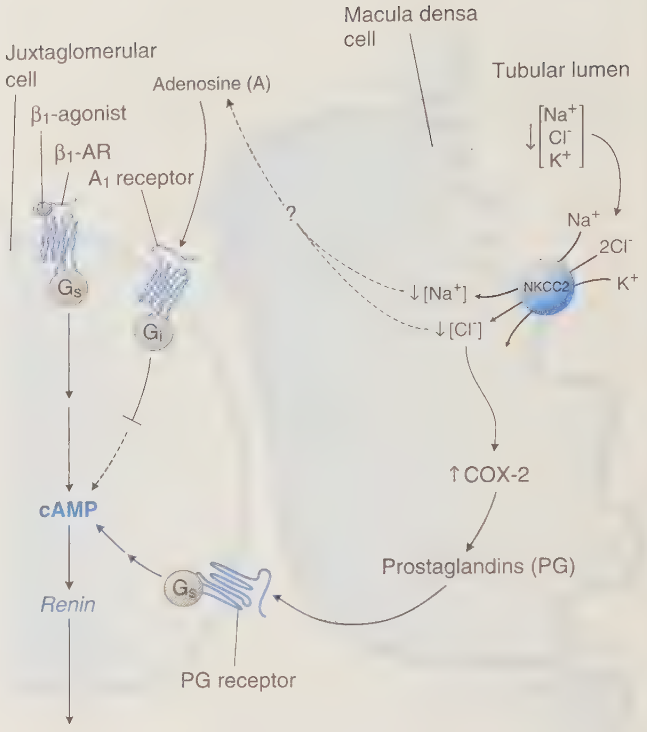

Macula densa
cell
Juxtaglomerular
cell
Tubular lumen
Adenosine (A)
β1-agonist
β1-AR
A1 receptor
↓[Na+]
[Cl-]
[K+]
Na+
2Cl-
K+
?
Gs
Gi
↓[Na+]
↓[Cl-]
NKCC2
↑COX-2
cAMP
Prostaglandins (PG)
Gs
Renin
PG receptor

**Figure 19-3. Modulation of Renin Release.** Renin is released by juxtaglomerular cells in response to diverse stimuli that signal volume depletion. First, decreased pressure in the afferent arteriole (not shown) stimulates increased renin release, possibly by releasing prostaglandins. Second, juxtaglomerular cells possess $\beta_1$-adrenergic receptors ($\beta_1$-AR) coupled to $G_s$, which stimulates adenylyl cyclase to increase the intracellular level of cAMP, which is a stimulus for renin release. Third, macula densa cells of the distal convoluted tubule modulate renin release based on the extent of distal tubule NaCl flow. In cases of decreased NaCl flux, the decreased $Cl^-$ entry through the $Na^+/2Cl^-/K^+$ transporter (NKCC2) on the macula densa cell apical membrane activates cyclooxygenase-2 (COX-2) activity, which increases prostaglandin production. These prostaglandins then diffuse to and activate juxtaglomerular cell prostaglandin (PG) receptors, which stimulate release of renin by increasing cAMP production. In contrast, increased cortical thick ascending limb (TAL) NaCl delivery leads, through still-debated mechanisms, to increased generation of adenosine in the juxtaglomerular mesangial interstitium. Activation of local, $G_i$-coupled juxtaglomerular cell $A_1$ receptors decreases intracellular cAMP, which is thought to transmit the signal to decrease renin release.

The above functions of AT II are mediated by its binding to the **AT II receptor subtype 1 ($AT_1$ receptor),** a G protein-coupled receptor. The actions of AT II are best understood in vascular smooth muscle cells, where $AT_1$ activates phospholipase C, leading to the release of $Ca^{2+}$ from intracellular stores and activation of protein kinase C. Inhibition of this receptor can lead to vascular smooth muscle cell relaxation and, hence, decrease systemic vascular resistance and blood pressure (see below). A second G protein-coupled AT II receptor, termed

$AT_2$, is expressed more prominently in fetal than in adult tissue. The $AT_2$ receptor seems to have a vasodilatory role.

## Natriuretic Peptides

Natriuretic peptides are hormones released by atria, ventricles, and vascular endothelium in response to volume overload. Three natriuretic peptides have been identified: A-type, B-type, and C-type natriuretic peptides. A-type natriuretic peptide **(ANP)** is released primarily by the atria, while B-type natriuretic peptide **(BNP)** is released mainly by the ventricles. C-type natriuretic peptide **(CNP)** is released by vascular endothelial cells.

*Natriuretic peptides are released in response to increased intravascular volume,* an effect that may be signaled by increased stretch of natriuretic peptide-secreting cells. Circulating natriuretic peptides bind to one of three receptors, termed the NPR-A, NPR-B, and NPR-C receptors. The NPR-A and NPR-B receptors are transmembrane proteins with intrinsic **guanylyl cyclase** activity (see Chapter 1, Drug–Receptor Interactions); activation of these receptors increases intracellular cGMP levels. The NPR-C receptor lacks an intracellular domain and may serve as a "decoy" or "buffer" receptor to reduce the level of circulating natriuretic peptide available to bind to signaling receptors. Both ANP and BNP bind with high affinity to NPR-A, while only CNP binds to NPR-B. All three natriuretic peptides bind to the NPR-C receptor (Fig. 19-4A).

Natriuretic peptides affect the cardiovascular system, the kidney, and the central nervous system. Integration of natriuretic peptide-derived signals serves to decrease volume overload and its sequelae. ANP relaxes vascular smooth muscle by increasing intracellular cGMP, which causes dephosphorylation of the myosin light chain and subsequent vasorelaxation (see Chapter 20). ANP also increases capillary endothelial permeability; this effect reduces blood pressure by favoring fluid filtration from the plasma into the interstitium (see Equation 19-1).

In the kidney, natriuretic peptides promote both increased glomerular filtration rate (GFR) and natriuresis. GFR is increased because of constriction of the efferent arteriole and dilation of the afferent arteriole, resulting in greater intraglomerular pressure and, therefore, increased plasma filtration. The natriuretic effects on the kidney result from antagonism of ADH action in the collecting ducts (see below) and antagonism of $Na^+$ reabsorption at multiple points along the distal nephron.

The central effects of natriuretic peptides are less well understood, but they include decreased perception of thirst (therefore decreasing fluid intake), decreased release of antidiuretic hormone (see below), and decreased sympathetic tone. The signaling mechanisms mediating these actions are uncertain, but may be via CNP, as this natriuretic peptide is expressed at high levels in the brain.

Although many of the effects of natriuretic peptides are still not understood completely, natriuretic peptides seem to play an important role in regulating situations of volume excess, including CHF. Much interest has recently focused on the relationship between heart failure and natriuretic peptides, and the physiology of natriuretic peptides continues to be refined.

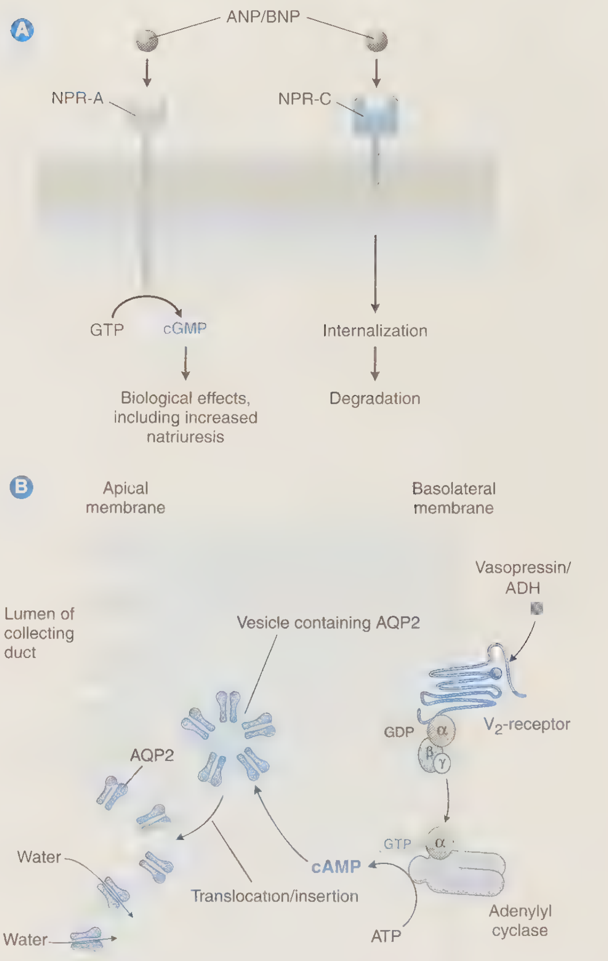


**Figure 19-4. Natriuretic Peptide and Antidiuretic Hormone Signaling Pathways.** **A.** A-type and B-type natriuretic peptides (ANP and BNP) are hormones secreted in response to volume overload. These peptides bind to natriuretic peptide receptor-A (NPR-A) and natriuretic peptide receptor-C (NPR-C). NPR-A is a transmembrane receptor with intrinsic guanylyl cyclase activity. Increased intracellular cGMP levels mediate the effects of natriuretic peptides, including increased natriuresis. NPR-C is believed to be a "decoy receptor," because the protein has no identified intracellular domain. Binding of natriuretic peptides to NPR-C may result in receptor internalization and degradation of natriuretic peptide bound to the receptor. A third natriuretic peptide, CNP, is expressed by vascular endothelial cells and binds to NPR-B (not shown). **B.** Antidiuretic hormone (ADH), also known as vasopressin, is secreted by the hypothalamus in response to increased osmolality and volume depletion. ADH mediates renal collecting duct water reabsorption by activation of the $G_s$-coupled $V_2$-receptor. Activation of $G_s$ leads to increased adenylyl cyclase activity and increased cAMP levels. cAMP increases collecting duct water reabsorption by increasing the translocation and insertion of aquaporin water channel (AQP2)-containing vesicles into the collecting duct apical membrane. The increased apical membrane AQP2 results in increased water flux across the collecting duct, and, therefore, greater reabsorption of filtered water. Hydrolysis of cAMP by phosphodiesterase leads to removal of AQP2 from the lumenal membrane by endocytosis of AQP2-containing vesicles (not shown).

## Antidiuretic Hormone

Antidiuretic hormone (**ADH,** also referred to as **vasopressin**) is a nonapeptide hormone secreted by the posterior pituitary gland in response to increased plasma osmolality or severe hypovolemia. ADH constricts the peripheral vasculature and promotes water reabsorption in the renal collecting duct. The actions are mediated by two distinct G protein-coupled receptors. The $V_1$ receptor, present in vascular smooth muscle cells, stimulates vasoconstriction through a $G_q$-mediated mechanism. The $V_2$ receptor, present in collecting duct principal cells, stimulates water reabsorption by a $G_s$-mediated mechanism (Fig. 19-4B). This $G_s$ signal increases cytosolic cAMP, which leads to activation of protein kinase A (PKA). PKA phosphorylates and activates proteins responsible for transport of aquaporin 2-containing vesicles to the apical membrane. The increased aquaporin 2 expression at the apical membrane promotes increased water reabsorption (see below). *Regulations of renal water reabsorption in the collecting duct modulates urine and plasma osmolality.* and serves as a reserve mechanism for increasing intravascular volume in situations of severe dehydration.

## Renal Sympathetic Nerves

Renal sympathetic nerves serve an important local regulatory feedback role to regulate renal blood flow. These sympathetic nerves innervate both afferent and efferent arterioles. In response to a decrease in intravascular volume, the renal sympathetic nerves decrease the GFR by stimulating constriction of the afferent arteriole to a greater degree than the efferent arteriole. This preferential constriction of the afferent arteriole decreases GFR, which ultimately decreases natriuresis. Renal sympathetic nerves also increase renin production by stimulation of $\beta_1$-adrenergic receptors on juxtaglomerular mesangial cells, and increase proximal tubule NaCl reabsorption.

## RENAL CONTROL OF NA$^+$ EXCRETION

Over the course of 24 hours, the kidneys filter approximately 180 L of fluid. For this reason, the neurohormonal mechanisms controlling extracellular volume status have important actions on the kidney. To increase or decrease body fluid volume, the kidneys must increase or decrease renal Na$^+$ reabsorption. Thus, an understanding of the renal control of Na$^+$ excretion is crucial to understanding the role of the kidney in regulation of body fluid volume.

The renal glomerulus produces an ultrafiltrate of plasma that flows through and is processed by the nephron, the functional unit of the kidney (Fig. 19-5). The postglomerular nephron is responsible for solute and water resorption from the filtrate, as well as for excretion of metabolic waste products and xenobiotics, including drugs. The renal tubular epithelial cells of the postglomerular nephron encircle a lengthy tubular lumen, the "urinary space," which leads to the ureters, urinary bladder, and urethra. The concentration of solutes in the glomerular ultrafiltrate is initially identical to that in the plasma. As the ultrafiltrate passes through the nephron, substrate-specific channels and transporters in the lumenal (or apical) membrane of polarized epithelial cells sequentially alter the filtrate solute concentrations. The function of these channels, in turn, is influenced by changes in solute concentrations in the cells themselves, as regulated in part by channels and transporters on the contralumenal (or basolateral) side of the cells. Systemic volume regulation by the kidney is accomplished by the integrated action of apical and basolateral ion channels and ion transporters and the accompanying reabsorption of water.

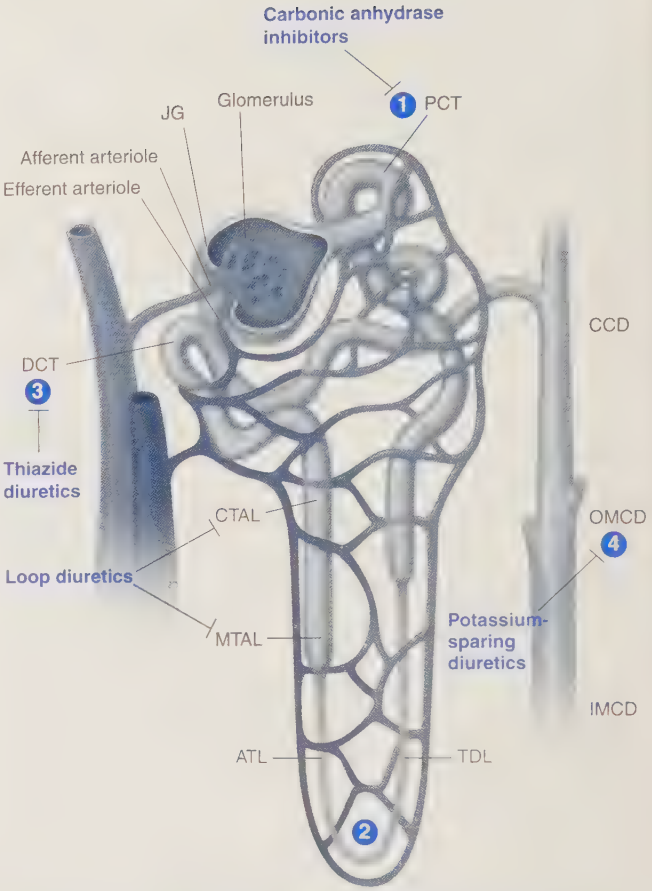


**Figure 19-5. Nephron Anatomy and Sites of Action of Diuretics.** Nephron fluid filtration begins at the glomerulus, where an ultrafiltrate of the plasma enters the renal epithelial (urinary) space. This ultrafiltrate then flows through four sequential regions of the nephron (1–4). 1. From the glomerulus, ultrafiltrate travels to the proximal convoluted tubule (PCT), then to the 2. Loop of Henle, which includes the thin descending limb (TDL), ascending thin limb (ATL), medullary thick ascending limb (MTAL) and cortical thick ascending limb (CTAL) of Henle. 3. The distal convoluted tubule (DCT) includes the macula densa and juxtaglomerular (JG) apparatus. 4. The collecting duct consists of the cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD). Pharmacologic agents inhibit specific solute transporters within each region. Carbonic anhydrase inhibitors act at the proximal convoluted tubule; loop diuretics act at the medullary and cortical thick ascending limbs; thiazide diuretics inhibit solute transport in the distal convoluted tubule; and potassium-sparing diuretics inhibit collecting duct Na$^+$ reabsorption.

The nephron beyond the glomerulus exhibits remarkable heterogeneity along its length. Four segments of the nephron are especially relevant to the pharmacology of body volume regulation (Fig. 19-5). These are the proximal tubule, the thick ascending limb (TAL) of the loop of Henle, the distal convoluted tubule, and the cortical collecting duct (CCD). In each tubular segment, a complex but tightly choreographed interplay of segment-specific ion transporters and/or channels collaborate in the reabsorption of NaCl across the lumen epithelium. NaCl reabsorption is key for water retention. Solute and water transport across each segment requires coordination of transport protein function in the lumenal and basolateral membranes. In addition, paracellular transport of ions across the tight junctions between cells requires regulation of cell-to-cell communication. Integration of transcellular and paracellular components of transepithelial transport requires integration of signals transmitted by sensors of extracellular and intracellular ion concentrations and of intracellular, local extracellular, and systemic volume. Alteration of ion transport by drugs in any nephron segment can induce compensatory regulation in more distal nephron segments.

## Proximal Tubule

The proximal tubule (PT) is the first reabsorptive site in the nephron and is responsible for approximately two-thirds of sodium reabsorption, 85–90% of bicarbonate reabsorption, and ~60% of chloride reabsorption (Fig. 19-6). Specific sodium-coupled symporters in the proximal tubule apical membrane drive renal reabsorption of glucose, amino acids, phosphate, and sulfate. The proximal tubule also mediates secretion and reabsorption of weak organic acids and bases coupled to sodium or proton symport or antiport, or to anion exchange mechanisms.

Bicarbonate reabsorption requires the coordinated action of apical and basolateral ion transporters together with apical and intracellular enzymatic activities (Fig. 19-6). At the lumenal surface of the proximal tubule, filtered bicarbonate encounters active proton secretion across the proximal tubule brush border microvilli. Two-thirds of the proton efflux is in exchange for influx of $Na^+$ via the **NHE3 $Na^+/H^+$ exchanger.** The remaining third of proton efflux is mediated by the vacuolar **$H^+$-ATPase (v$H^+$ ATPase).**

$HCO_3^-$ permeability of the lumenal membrane is low. However, the outer leaflet of the lumenal membrane of the proximal tubular cell harbors the glycosylphosphatidylinositol-linked exo-enzyme **carbonic anhydrase IV (CAIV).** CAIV converts lumenal $HCO_3^-$ to $CO_2$ and $OH^-$. The $OH^-$ is rapidly hydrated to water by the abundance of local protons, and the $CO_2$ freely diffuses into the cytoplasm of the proximal tubular epithelial cell. The intracellular $CO_2$ is rapidly rehydrated by cytoplasmic **carbonic anhydrase II (CAII)** to $HCO_3^-$, a reaction that consumes intracellular $OH^-$ accumulated as a result of the $H^+$-extruding activities of apical NHE3 and v$H^+$ ATPase. The $HCO_3^-$ produced by the CAII reaction is then transported across the basolateral membrane of the epithelial cell by cotransport with the $Na^+$ that crossed the apical membrane of the cell via NHE3, for a net reabsorption of sodium and bicarbonate. The $Na^+/HCO_3^-$ cotransporter **NBC1** mediates electrogenic basolateral efflux of 3 $HCO_3^-$ ions with each cotransported $Na^+$ ion. Basolateral $K^+$ channels maintain an inside-negative membrane potential to enhance the driving force for net efflux of two negative charges per NBC1 transport cycle. Emerging evidence suggests the presence of several distinct forms of carbonic anhydrase at the extracellular surface of the basolateral membrane that help dissipate local accumulation of bicarbonate within the small interstitial space between epithelial cells and peritubular capillaries.

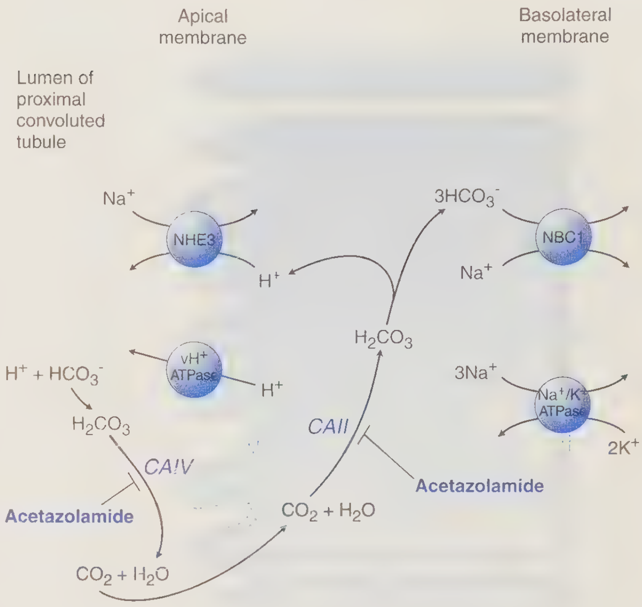


**Figure 19-6. Proximal Convoluted Tubule Cell.** A significant percentage of proximal convoluted tubule $Na^+$ is reabsorbed via the NHE3 $Na^+/H^+$ antiporter. The action of this antiporter, together with an apical membrane vacuolar ATPase (v$H^+$ ATPase), results in significant $H^+$ extrusion into the proximal convoluted tubule urinary space. $H^+$ extrusion is coupled to $HCO_3^-$ reabsorption by the action of an apical membrane carbonic anhydrase IV (CAIV) that catalyzes the cleavage of $HCO_3^-$ into $OH^-$ and $CO_2$. $OH^-$ combines with $H^+$ to form water, while $CO_2$ diffuses into the nephron epithelial cell cytoplasm. The cytoplasmic enzyme carbonic anhydrase II (CAII) catalyzes formation of $HCO_3^-$ from $CO_2$ and $OH^-$; the $HCO_3^-$ is then extruded into the interstitium together with $Na^+$. The net result of this process is reabsorption of $HCO_3$ with $Na^+$ by the basolateral cotransporter NBC1. Acetazolamide inhibits both isoforms of carbonic anhydrase; the decreased carbonic anhydrase activity results in decreased $Na^+$ and $HCO_3^-$ absorption.

Solute absorption in the proximal tubule is iso-osmotic—water accompanies reabsorbed ions to maintain osmotic balance. In the past, water flow was assumed to be largely paracellular. However, recent data from mice genetically modified to lack the **aquaporin** water channel AQP1 (and from rare cases of humans lacking AQP1) demonstrate that most proximal tubular water reabsorption is transcellular. This seems to hold true for all water-permeable nephron segments.

## Thick Ascending Limb of the Loop of Henle

The tubular fluid emerging from the thin ascending limb is hypertonic and has an elevated NaCl concentration. Reabsorption of NaCl without accompanying water by the **thick ascending limb** (TAL) dilutes the tubular fluid (Fig. 19-7). It also provides, together with urea reabsorption, the interstitial solute required for the countercurrent concentrating mechanism that generates and maintains the corticomedullary osmotic gradient of the kidney. The TAL reabsorbs between 25%

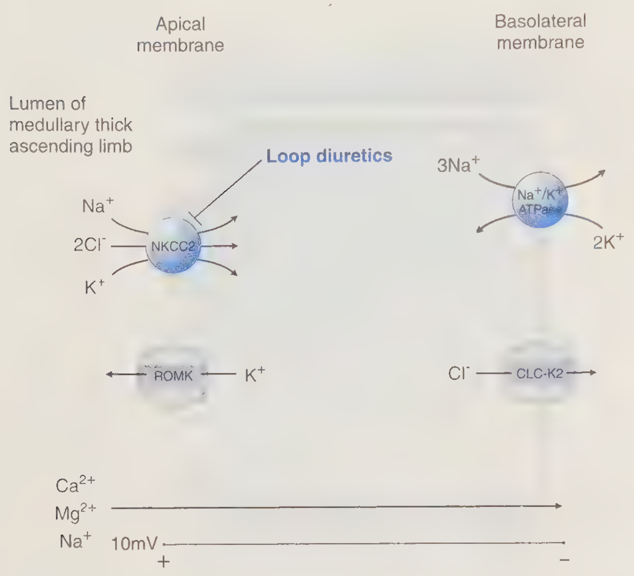


**Figure 19-7. Medullary Thick Ascending Limb Cell.** The medullary thick ascending limb of the loop of Henle absorbs Na$^+$ through an apical membrane Na$^+$/K$^+$/2Cl$^-$ (NKCC2) transporter. The Na$^+$/K$^+$ ATPase pumps sodium from the cytoplasm into the interstitium, and a basolateral Cl$^-$ channel (CLC-K2) transports Cl$^-$ into the interstitium. K$^+$ is primarily recycled into the urinary space via a lumenal K$^+$ channel (ROMK). The combined activities of apical ROMK and basolateral CLC-K2 result in a lumen-positive transepithelial potential difference (approximately 10 mV) that drives paracellular absorption of cations, including Ca$^{2+}$ and Mg$^{2+}$. Loop diuretics inhibit NKCC2, resulting in significantly increased renal sodium excretion. Disruption of the positive transepithelial potential by loop diuretics increases the excretion of Ca$^{2+}$ and Mg$^{2+}$.

and 35% of the filtered Na$^+$ load by means of the lumenal membrane Na$^+$-K$^+$-2Cl$^-$ cotransporter, **NKCC2.** The Cl$^-$ imported by NKCC2 exits the basolateral side of the cell via **ClC-K2** chloride channels. The Na$^+$ imported from the lumen via NKCC2 leaves the basolateral side of the cell via the Na$^+$/K$^+$-ATPase. Because Cl$^-$ carries a negative charge, exit of Cl$^-$ through ClC-K2 depolarizes the cell. The stoichiometry of the Na$^+$/K$^+$ ATPase, 3Na$^+$ out per 2 K$^+$ in, partly counters this depolarization, but full repolarization of the cell is accomplished by the apical K$^+$ channel **ROMK,** which recycles back into the lumen the K$^+$ imported into the cell from the lumen via NKCC2. The coordinated operation of these apical and basolateral transporters and channels generates a lumen-positive potential across the TAL. This transepithelial potential difference drives the paracellular reabsorption of additional Na$^+$ from lumen to interstitium to provide transepithelial charge balance. This paracellular component of Na$^+$ reabsorption, ~50% of the Na$^+$ reabsorbed by the TAL, effectively reduces energetic cost to the TAL (measured as ATP consumption) by 50% because Na$^+$/K$^+$ transport consumes most of the TAL cell's ATP. Even with the energy conserved by this paracellular Na$^+$ absorptive pathway, the TAL working at maximal capacity can consume up to 25% of the body's total ATP consumption, ~65 moles per day at rest. The lumen-positive transepithelial potential of the TAL also drives paracellular reabsorption of calcium and magnesium ions.

## Distal Convoluted Tubule

This continuation of the diluting segment actively reabsorbs from 2% to 10% of the filtered NaCl load, while remaining impermeable to lumenal water (Fig. 19-8). Lumenal Na$^+$ enters the epithelial cells of the distal convoluted tubule via electroneutral, K$^+$-independent cotransport of NaCl via the **NCC1** Na$^+$-Cl$^-$ cotransporter. Basolateral exit of the imported Na$^+$ is mediated by Na$^+$/K$^+$-ATPase, while the imported Cl$^-$ exits by poorly defined basolateral anion pathways that likely include both electrogenic Cl$^-$ channels and electroneutral K$^+$-Cl$^-$ cotransport. The distal convoluted tubule (DCT) also mediates transepithelial reabsorption of lumenal calcium via regulated apical calcium channels and lumenal magnesium via apical magnesium channels.

## Collecting Duct

This terminal portion of the nephron is divided into **cortical, outer medullary,** and **inner medullary** collecting duct segments (Fig. 19-9). The more proximal portions of the collecting duct (CD) consist of two cell types, **principal cells** and **intercalated cells.** Principal cells reabsorb between 1% and 5% of filtered sodium load, depending on plasma aldosterone levels (aldosterone increases sodium reabsorption and water retention, see below). Lumenal Na$^+$ enters the principal cells of the cortical collecting duct via the heterotrimeric epithelial Na$^+$ channels, **ENaC,** of the apical membrane.

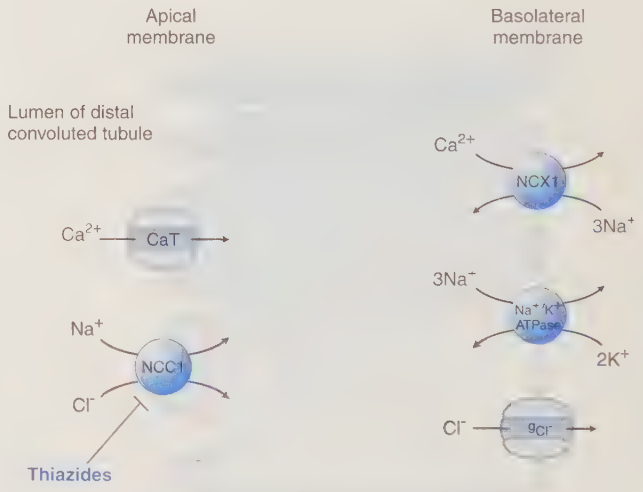


**Figure 19-8. Distal Convoluted Tubule Cell.** Distal convoluted tubule cells absorb Na$^+$ via an apical membrane NaCl cotransporter (NCC1). Na$^+$ is then transported across the basolateral membrane into the interstitium via the Na$^+$/K$^+$ ATPase, and Cl$^-$ is transported from the cytosol into the interstitium via Cl$^-$ channels (g$_{Cl^-}$) and perhaps by K$^+$-Cl$^-$ cotransport (not shown). Renal epithelial cells of the distal convoluted tubule also absorb Ca$^{2+}$ via apical membrane Ca$^{2+}$ channels (CaT), and Ca$^{2+}$ is transported across the basolateral membrane into the interstitium by the Na$^+$/Ca$^{2+}$ exchanger NCX1 and by the Ca$^{2+}$ channel PMCA (not shown). Thiazides inhibit NCC1, resulting in increased Na$^+$ excretion. Thiazides also increase epithelial cell absorption of Ca$^{2+}$ by an unknown mechanism (not shown).

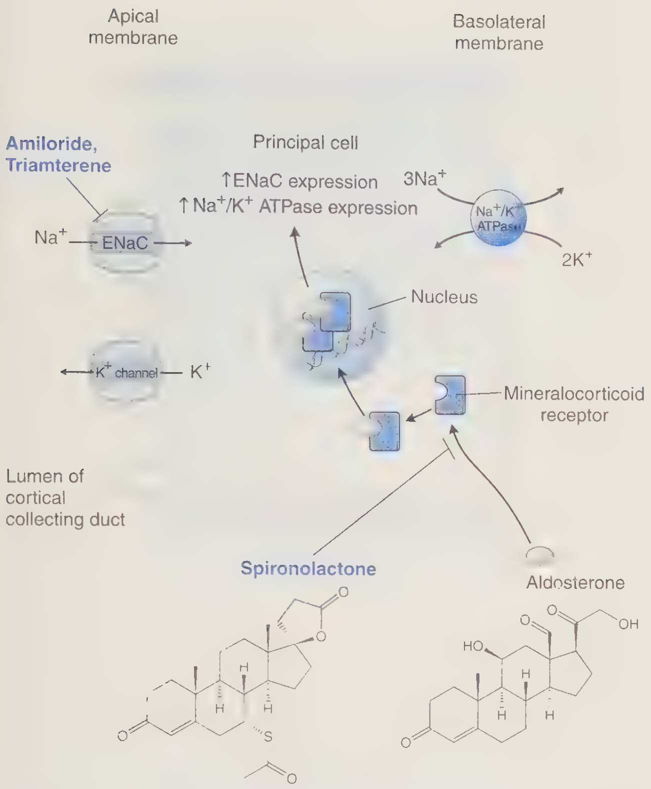


**Figure 19-9. Cortical Collecting Duct Principal Cell.** Cortical collecting duct principal cells absorb Na$^+$ via an apical membrane Na$^+$ channel (ENaC). Cytoplasmic Na$^+$ is then transported across the basolateral membrane via the Na$^+$/K$^+$ ATPase. In addition, collecting duct cells express apical membrane K$^+$ channels that allow K$^+$ to exit into the urinary space. ENaC expression and apical surface localization is modulated by aldosterone. Aldosterone binds to the mineralocorticoid receptor, which then increases transcription of the gene encoding ENaC, as well as genes encoding other components of Na$^+$ transport. The collecting duct principal cell is the site of action of the two classes of potassium-sparing diuretics. Mineralocorticoid receptor antagonists such as spironolactone competitively inhibit the interaction of aldosterone with the mineralocorticoid receptor, and therefore decrease expression of ENaC. Direct inhibitors of ENaC, such as amiloride and triamterene, inhibit Na$^+$ influx through the ENaC channel.

Intracellular Na$^+$ exits the basolateral side of the cell via the Na$^+$/K$^+$-ATPase. Principal cells also secrete K$^+$ into the lumen to maintain tight control of plasma [K$^+$], as well as to minimize the transepithelial potential difference resulting from Na$^+$ reabsorption. In addition, cortical and outer medullary principal cells, as well as cells of the inner medullary collecting duct, exhibit vasopressin (ADH)-responsive water channels. ADH activates water reabsorption by promoting, via binding and activating the basolateral membrane $G_s$ protein-coupled $V_2$ receptor, reversible insertion into the apical membrane of intracellular vesicles that contain aquaporin 2 (AQP2) water channels (Fig. 19-4B). At least two subtypes of intercalated cells contribute to systemic acid-base balance by secreting either bicarbonate or protons. Intercalated cells may also regulate Cl$^-$ absorption via anion exchangers, K$^+$ absorption by electroneutral lumenal H$^+$/K$^+$ exchange ATPases, and $NH_4^+$ secretion by proteins related to the erythroid Rhesus (Rh) antigens.

## PATHOPHYSIOLOGY OF EDEMA FORMATION

Edema is defined as the *accumulation of fluid in the interstitial space.* Edema can be either exudative (having a high protein content) or transudative (having a low protein content, essentially a plasma ultrafiltrate). Exudative edema occurs as part of the acute inflammatory response (see Chapter 39, Principles of Inflammation and the Immune System). The type of edema considered here is **transudative edema,** which can result from pathologic renal retention of Na$^+$.

Under physiologic conditions, any increased fluid filtration across the capillary membrane is quickly counterbalanced by homeostatic mechanisms. This return to a physiologic set point is mediated by three factors: osmotic forces, lymphatic drainage, and long-term modulation of volume by physiologic sensors and signals. Osmotic forces play an immediate role in fluid shifts between compartments. For example, increased fluid shift to the interstitial space will result in increased interstitial hydrostatic pressure and increased plasma oncotic pressure. Both of these variables favor fluid shift back into the intravascular space (Fig. 19-1). The lymphatic system can also increase return of filtered fluid dramatically, thereby decreasing the amount of any filtered fluid that remains in the interstitial space. Over a period of days to weeks, volume sensors and signals respond to changes in volume by altering the extent of natriuresis or sodium reabsorption necessary to maintain a constant intravascular volume. These combined systems closely monitor and regulate intravascular volume. Therefore, *the pathophysiology of transudative edema formation almost always requires an element of pathologic renal Na$^+$ retention.*

The three most common clinical situations resulting in edema formation are CHF, cirrhosis, and nephrotic syndrome. All of these diseases manifest deranged Na$^+$ reabsorption caused by pathologic alterations in volume regulation. Understanding the pathophysiology of edema formation in these diseases provides a rationale for therapeutic use of natriuretic agents.

## CONGESTIVE HEART FAILURE

Heart failure occurs in any situation where the heart is unable to perfuse distal tissues and organs adequately. The inadequate perfusion leads to a decreased forward flow of blood with consequent venous congestion. The "congestive" aspect of heart failure is used to describe the increased edema formation, especially pulmonary edema, that can occur in acute situations where the increased venous pressure, secondary to decreased cardiac output, creates a dramatically increased capillary hydrostatic pressure that favors transudation of fluid into the interstitial space. In the introductory case, Mr. Reilly's compromised cardiac function led to increased pulmonary venous congestion and peripheral edema; the pulmonary congestion was responsible for his sensation of dyspnea. The pathophysiology of congestive heart failure is discussed in further detail in Chapter 23; the discussion here focuses only on edema formation.

The fundamental cause of increased Na$^+$ retention in congestive heart failure (CHF) is *perceived volume depletion*

(Fig. 19-10). The inadequate arterial blood flow is perceived by high-pressure volume receptors, including the juxtaglomerular apparatus, as a decrease in intravascular volume. The kidney, therefore, increases production of renin, leading to increased angiotensin II (AT II) production and secretion of aldosterone by the adrenal cortex. AT II and aldosterone both increase renal Na$^+$ absorption, by mechanisms described above. Other important mediators of increased renal Na$^+$ reabsorption may include endothelin, prostaglandins, and renal sympathetic nerves; these pathways act to maintain renal perfusion pressure and glomerular filtration fraction in the presence of perceived volume depletion.

Under physiologic conditions, low-pressure systems such as neural responses and natriuretic peptides sense the increased pressure resulting from venous congestion and, therefore, promote natriuresis. This response limits the extent of renal Na$^+$ reabsorption and prevents pathologic extracellular fluid volume expansion. However, both neural and natriuretic peptide signaling pathways are disrupted in CHF. The altered neural signaling present in CHF activates excessive sympathetic responses, in part to increase norepinephrine-induced inotropy of the ventricles, thereby augmenting ejection fraction and maintaining forward flow. This increased sympathetic activity blunts any parasympathetic response that would otherwise decrease renal Na$^+$ retention. Plasma natriuretic peptide is significantly increased in CHF, but experimental evidence suggests that there may exist an end-organ resistance to this hormone and, therefore, a blunted natriuretic response to increases in circulating hormone.

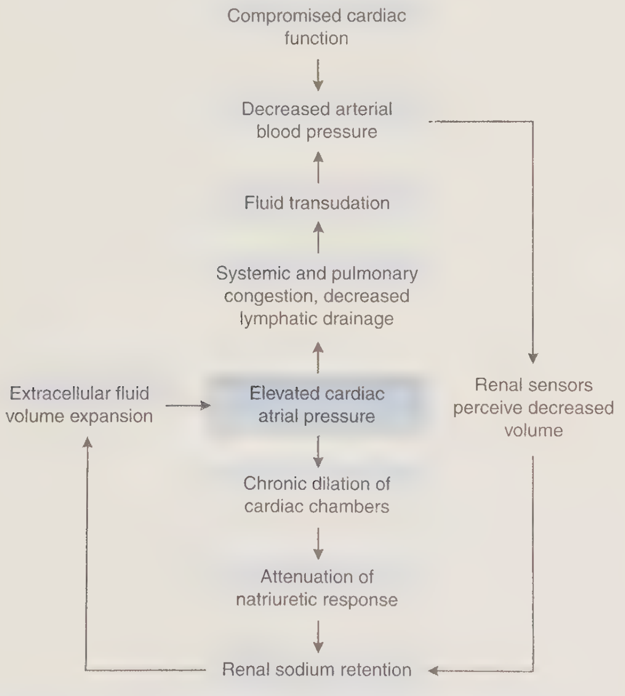

Compromised cardiac function
Decreased arterial blood pressure
Fluid transudation
Systemic and pulmonary congestion, decreased lymphatic drainage
Extracellular fluid volume expansion
Elevated cardiac atrial pressure
Renal sensors perceive decreased volume
Chronic dilation of cardiac chambers
Attenuation of natriuretic response
Renal sodium retention

**Figure 19-10. Mechanisms of Na$^+$ Retention in Congestive Heart Failure.** In congestive heart failure, compromised cardiac function leads to decreased arterial blood pressure and subsequent activation of renal volume sensors. These sensors activate renal sodium retention to expand extracellular volume and, therefore, correct the decreased arterial blood pressure. The expansion of extracellular volume increases cardiac atrial pressure. In the failing heart, the increased fluid cannot be pumped forward, and instead increases hydrostatic pressure in the pulmonary and systemic circuits, leading to fluid transudation and development of edema. In addition, evidence suggests that chronic dilation of cardiac chambers leads to local resistance to natriuretic peptide action; in the absence of an appropriate natriuretic response, the kidney continues reabsorbing Na$^+$ despite increased extracellular volume.

Diuretics and ACE inhibitors have found significant application in the interruption of CHF pathophysiology. As discussed below, diuretics decrease renal Na$^+$ reabsorption and, thereby, reduce the extracellular volume expansion that provides a stimulus for edema formation. As demonstrated in the introductory case, diuretics can be used in an acute setting to reduce the extent of pulmonary edema. Over the long term, decreased Na$^+$ retention also affects afterload by reducing intravascular volume and, hence, systemic blood pressure and systolic ventricular pressure. ACE inhibitors may interrupt pathologic paracrine signaling pathways that otherwise lead to deterioration of cardiac tissue and worsening of CHF (see below).

## CIRRHOSIS

Chronic inflammation of the liver can lead to **cirrhosis,** a disease characterized by hepatocellular injury leading to fibrosis of the hepatic parenchyma. Fibrosis alters hepatic hemodynamics by obstructing venous outflow from the liver and, as a result, increasing hydrostatic pressure in the portal vein. The obstruction to flow causes increased portosystemic shunting of blood away from the liver and into the systemic circulation. Hepatocellular injury disrupts the synthetic and metabolic capabilities of the liver, leading to decreased production of albumin, clotting factors, and numerous other hormones and prohormones.

The mechanism of renal Na$^+$ retention in cirrhosis is unknown, but two theories have been proposed (Fig. 19-11). The **underfill theory** (Fig. 19-11A) proposes that obstruction of hepatic venous outflow leads to an increase in intrahepatic hydrostatic pressure. The increased hydrostatic pressure causes increased fluid transudation across the hepatic sinusoids and, therefore, increased lymphatic flow through the thoracic duct. Under physiologic conditions, the lymphatic system is able to increase its flow dramatically and therefore limit the extent of interstitial fluid accumulation. In cirrhosis, however, lymphatic flow can exceed 20 L/day, eventually overwhelming the ability of the lymphatic system to return transudate to the systemic circulation and leading to the formation of **ascites** (accumulation of serous fluid in the abdominal cavity). The ascites formation decreases intravascular volume, because fluid is shunted from the plasma into the abdominal cavity. The decreased intravascular volume leads to a decrease in cardiac output, with subsequent activation of baroreceptors that activate renal Na$^+$ retention. Thus, the underfill model is conceptually similar to the mechanism of edema formation in CHF, in that the kidney initiates Na$^+$ reabsorption in response to the perceived decrease in intravascular volume.

The **overflow model** of ascites formation postulates that ascites formation involves an element of primary renal Na$^+$ retention. (Fig. 19-11B) In this model, postsinusoidal obstruction activates the **hepato–renal reflex,** an incompletely characterized autonomic response that results in increased

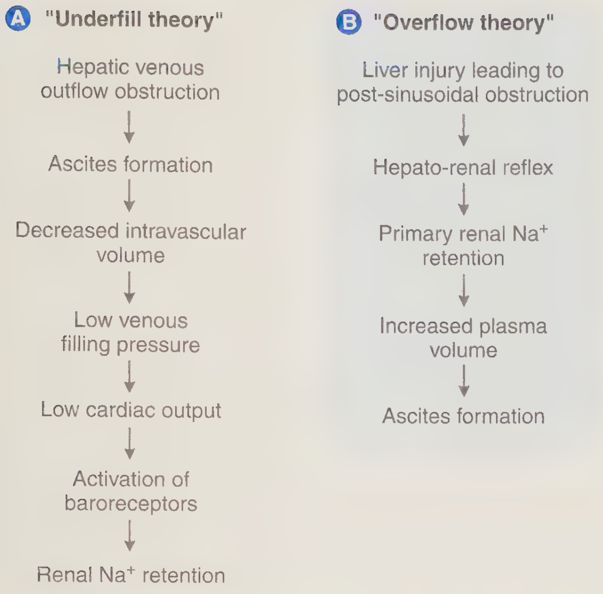

A "Underfill theory"
Hepatic venous outflow obstruction
Ascites formation
Decreased intravascular volume
Low venous filling pressure
Low cardiac output
Activation of baroreceptors
Renal $Na^+$ retention
B "Overflow theory"
Liver injury leading to post-sinusoidal obstruction
Hepato-renal reflex
Primary renal $Na^+$ retention
Increased plasma volume
Ascites formation

**Figure 19-11. Proposed Mechanisms of $Na^+$ Retention in Cirrhosis.** The postsinusoidal obstruction in cirrhosis is associated with accumulation of ascites fluid. Two theories exist to explain the mechanism of ascites formation. In (**A**), hepatic venous outflow obstruction causes increased hydrostatic pressure, which initiates ascites formation. The increased ascites decreases intravascular volume, leading to low venous filling pressure, decreased cardiac output, and subsequent activation of arterial baroreceptors that initiate renal $Na^+$ retention. In (**B**), postsinusoidal obstruction activates the hepato-renal reflex, an autonomic response between the liver and kidney that initiates renal $Na^+$ reabsorption by a poorly understood mechanism. The increased renal $Na^+$ retention leads to an expansion of plasma volume, increased hydrostatic pressure in the portal circuit, and the formation of ascites.

renal $Na^+$ retention. Pathologic retention of $Na^+$ leads to intravascular volume expansion, increased portal hydrostatic pressure, and formation of ascites. Although not well understood, this mechanism is consistent with a number of model systems demonstrating that renal $Na^+$ retention in cirrhosis occurs before the development of ascites.

In reality, the formation of ascites may involve elements of both the underfill and overflow theories. Both theories begin with the observation that cirrhosis leads to significant hepatic outflow obstruction, and both must consider compromised portal hemodynamics, decreased hepatic synthetic and secretory functions leading to decreased plasma oncotic pressure, and poorly characterized interactions between the liver and the kidney. Elucidation of the mechanisms of the hepato-renal reflex may lead in the future to effective pharmacologic interventions to manage the development of ascites in cirrhosis.

## NEPHROTIC SYNDROME

Nephrotic syndrome is a clinical diagnosis characterized by massive proteinuria (>3.5 g/day), hypercholesterolemia, hypoalbuminemia, and edema. There are numerous causes for nephrotic syndrome, including immune complex diseases of the glomerulus, diabetes, lupus, and amyloidosis.

Classically, edema formation in nephrotic syndrome has been described to occur by the following sequence: First, massive proteinuria leads to decreased plasma oncotic pressure. Second, decreased plasma oncotic pressure reduces the forces favoring fluid retention in the capillary and, therefore, an increased amount of transudate moves to the interstitium. Third, increased net fluid transudation causes a decrease in intravascular volume. Fourth, the decreased intravascular volume activates volume sensors to increase renal $Na^+$ retention. Fifth, the consequent expansion in fluid volume, in the absence of compensatory albumin synthesis, maintains low plasma oncotic pressure and, therefore, continued edema formation. In this view, renal $Na^+$ retention is secondary to decreased renal arterial perfusion. However, experimental evidence suggests that the edema in nephrotic syndrome may also be caused by an element of primary renal $Na^+$ retention. It has been suggested that the primary $Na^+$ retention in nephrotic syndrome may be localized to the distal tubule, and may result from resistance to natriuretic peptides, or increased sympathetic nervous system activity.

Because the pathophysiology of edema formation in nephrotic syndrome is likely linked more to hypoalbuminemia than renal $Na^+$ retention, treatment of nephrotic syndrome typically consists of correction of the underlying renal disorder, which leads to a decrease in proteinuria and eventually correction of the edema. Diuretics are used in the short term to minimize edema formation.

# PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic modulation of extracellular fluid volume can be divided into agents that modify neurohormonal volume signals and agents that act directly on the nephron segments to alter renal $Na^+$ handling. The former category includes agents that interrupt the renin–angiotensin axis, alter circulating levels of natriuretic peptides, or interrupt ADH signaling. The latter category includes the various classes of diuretics, which directly target renal ion transporter channel function or expression to increase renal $Na^+$ excretion, with consequent decrease in extracellular fluid volume. Some overlap exists between these two categories, as the neurohormonal volume signals, like the diuretics, possess many direct (although less well understood) actions on nephron segments.

## AGENTS THAT MODIFY VOLUME SIGNALS

### Inhibitors of the Renin–Angiotensin System

There are three pharmacologic strategies for interruption of the renin-angiotensin-aldosterone system. First, ACE inhibitors interrupt the conversion of angiotensin I to angiotensin II. Second, angiotensin receptor antagonists are competitive antagonists of the $AT_1$ receptor, and thus inhibit the target-organ effects of angiotensin II. Third, inhibitors of the mineralocorticoid receptor block aldosterone action at the nephron collecting duct. The first two classes of agents are discussed here; because inhibitors of aldosterone action are considered diuretics, these agents are addressed below (see "Potassium-Sparing Diuretics").

### 1. Angiotensin Converting Enzyme Inhibitors

Pharmacologic interruption of the renin–angiotensin axis is most commonly achieved through inhibition of AT II generation via inhibition of angiotensin converting enzyme (ACE). Because AT II is the primary mediator of the activity of the renin-angiotensin–aldosterone system, decreased conversion of AT I to AT II inhibits arteriolar vasoconstriction, decreases aldosterone synthesis, inhibits renal proximal tubule NaCl reabsorption, and decreases ADH release. In addition, because ACE proteolytically cleaves many proteins, including bradykinin, ACE inhibitors also increase bradykinin levels. Bradykinin causes smooth muscle relaxation by binding to bradykinin receptors on the endothelial cell surface, leading to $Ca^{2+}$ mobilization, eNOS activation, and increased NO production (see Chapter 20). All of these actions result in decreased blood pressure and increased natriuresis. Thus, ACE inhibitors decrease blood pressure primarily by decreasing AT II levels and secondarily by increasing bradykinin levels (Fig. 19-12).

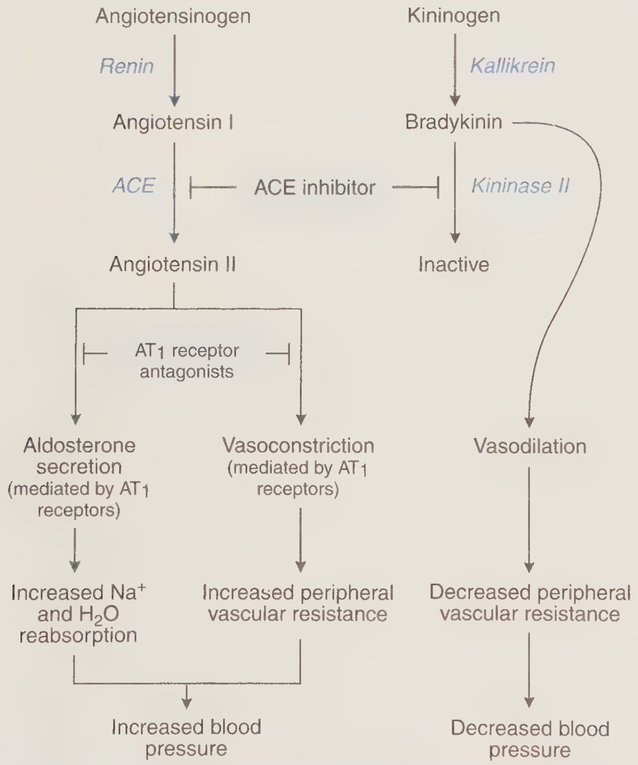

Angiotensinogen
Kininogen
Renin
Kallikrein
Angiotensin I
Bradykinin
ACE
ACE inhibitor
Kininase II
Angiotensin II
Inactive
AT1 receptor antagonists
Aldosterone secretion (mediated by AT1 receptors)
Vasoconstriction (mediated by AT1 receptors)
Vasodilation
Increased Na+ and H2O reabsorption
Increased peripheral vascular resistance
Decreased peripheral vascular resistance
Increased blood pressure
Decreased blood pressure

**Figure 19-12. Effects of Renin–Angiotensin System Antagonists on Blood Pressure.** ACE inhibitors prevent the conversion of angiotensin I to angiotensin II (both in the lung and locally in blood vessels and tissues) and inhibit the inactivation of bradykinin. Both actions of ACE inhibitors lead to vasodilation. The inhibition of angiotensin II formation decreases $AT_1$-mediated vasoconstriction and decreases aldosterone secretion; both of these effects act to decrease blood pressure. The inhibition of kininase II activity results in higher bradykinin levels, which promote vasodilation. The increased vasodilation decreases peripheral vascular resistance, which decreases blood pressure. In contrast, $AT_1$ antagonists (also known as angiotensin receptor blockers, or ARBs) decrease aldosterone synthesis and interrupt $AT_1$-mediated vasoconstriction, but do not alter bradykinin levels. Note that a bradykinin-induced cough is a major side effect of ACE inhibitors, but not of $AT_1$ antagonists.

The contribution of reduced plasma aldosterone levels to the antihypertensive effects of ACE inhibitors remains unclear. This uncertainty is related to the fact that the renal vasoconstrictive effects of angiotensin II occur primarily at the efferent arteriole of the glomerulus. A preferential decrease in efferent relative to afferent arteriolar tone will reduce intraglomerular pressure, resulting in decreased GFR. This reduction of GFR may counterbalance the anticipated reduction of $Na^+/H_2O$ retention that should occur as a result of ACE inhibitor-induced reduction of aldosterone levels.

The twelve ACE inhibitors currently approved by the FDA all act by the same mechanism. **Captopril** is the prototypical ACE inhibitor. ACE inhibitors exhibit three patterns of metabolism. Captopril represents the first pattern: It is active as administered but also processed to an active metabolite. The second and most common pattern, exemplified by **enalapril** and **ramipril,** consists of ester prodrugs that are converted in the plasma to active metabolites. The active forms of each of these drugs are denoted by an extra "-at" at the end of the drug name; enalaprilat and ramiprilat are the active forms of enalapril and ramipril, respectively. **Lisinopril** is the lone example of the third pattern, in which the drug is administered in active form and excreted unchanged by the kidneys. Captopril, enalapril, ramipril, and lisinopril have all been studied in large-scale clinical trials.

Although ACE inhibitors are generally well tolerated, important adverse effects of these agents include **cough** and **angioedema** caused by potentiation of bradykinin's effects. The cough, occurring in up to 20% of patients taking captopril, is usually dry and nonproductive. While not causing any serious physiologic effects, the cough may decrease quality of life and limit patient compliance. Angioedema, which can occur in 0.1–0.2% of patients, is a potentially life-threatening cause of airway obstruction. This side effect almost always occurs within the first week of initiating therapy and may require emergency intervention.

ACE inhibitors may precipitate first-dose **hypotension** and/or **acute renal failure,** and, thus, are administered at low initial dose. These side effects are more common in patients with bilateral renal artery stenosis. In such patients, renal function can be dependent on increased angiotensin II activity, because elevated angiotensin II maintains GFR by preferential efferent arteriolar constriction. For this reason, bilateral renal artery stenosis is a contraindication to ACE inhibitor therapy. ACE inhibitors interrupt aldosterone synthesis, and so can produce **hyperkalemia.** Hyperkalemia is more commonly observed when ACE inhibitors are used in conjunction with potassium-sparing diuretics (see below) such as spironolactone, amiloride, and triamterene.

ACE inhibitors have important clinical applications in the treatment of hypertension, heart failure, acute myocardial infarction, and chronic renal failure. In many cases, ACE inhibitors are increasingly considered first-line agents for hypertension, especially when a patient has concomitant left ventricular wall dysfunction or diabetes (see Chapter 23). ACE inhibitors have broad applicability to all forms of hypertension, including hypertension where there is no clear increase in plasma renin levels. Long-term use of ACE inhibitors retards progression of the cardiac contractile dysfunction observed in congestive heart failure and after myocardial infarction, by little understood mechanisms that may involve inhibition of paracrine hormones that stimulate pathologic tissue hypertrophy. ACE inhibitors can also delay progression of diabetic nephropathy. This effect may reflect attenuation of renal paracrine signaling pathways, with a consequent improvement in renal hemodynamics.

### 2. *Angiotensin Receptor Antagonists*

$AT_1$ receptor antagonists, such as **losartan** and **valsartan,** inhibit the action of AT II at its receptor. Compared to ACE inhibitors, $AT_1$ receptor antagonists may allow more complete inhibition of AT II's actions, because ACE is not the only enzyme that can generate AT II. Also, $AT_1$ receptor antagonists have no effect on bradykinin metabolism and, thus, are thought to minimize the side effects of cough and angioedema. However, the inability of $AT_1$ receptor antagonists to potentiate bradykinin's vasodilatory effects may result in less effective vasodilation. In addition, $AT_1$ receptor antagonists may increase $AT_2$ receptor activity indirectly, because the AT II, unable to bind to the $AT_1$ receptor, could preferentially interact with $AT_2$ receptors.

$AT_1$ receptor antagonists are approved for treatment of hypertension and are under study for treatment of heart failure. The recent CHARM trials have suggested that $AT_1$ receptor antagonists, in combination with ACE inhibitors, provide mortality benefit in severe heart failure. $AT_1$ receptor antagonists are currently considered second-line agents for hypertension and are prescribed when a patient has an intolerable adverse reaction (such as cough) to ACE inhibitors. Studies comparing the efficacy of ACE inhibitors versus $AT_1$ receptor antagonists in the treatment of hypertension and renal disease are currently underway.

## B-Type Natriuretic Peptide

Recently, **nesiritide,** a recombinant human sequence B-type natriuretic peptide (BNP), was approved for short-tem management of decompensated congestive heart failure. Because nesiritide is a peptide, it is ineffective when given orally. In clinical trials, administration of nesiritide was shown to decrease pulmonary capillary wedge pressure (a measure of hydrostatic pressure in the pulmonary system), decrease systemic vascular resistance, and improve cardiac parameters such as stroke volume. Thus, nesiritide seems to improve hemodynamics in acute CHF. Although nesiritide was not shown to be more efficacious than dobutamine (one of the common agents used in the treatment of decompensated CHF; see Chapter 23), nesiritide may be associated with a lower incidence of arrhythmias than dobutamine.

**Hypotension** was the major side effect of nesiritide during clinical trials; this side effect is a logical extension of the vasorelaxant properties of natriuretic peptides. Co-administration of nesiritide with ACE inhibitors was associated in these trials with an increased risk of hypotension.

## Antidiuretic Hormone (ADH) Antagonists

There are no selective ADH antagonists in clinical use. The tetracycline analogue **demeclocycline** is an ADH antagonist used in the context of syndromes of inappropriate ADH secretion (SIADH), when dietary water restriction is not feasible.

# AGENTS THAT DECREASE RENAL NA+ REABSORPTION

As discussed above, the kidney modifies the ionic composition of the glomerular filtrate by the concerted action of ion transporters and channels in both apical and basolateral membranes of renal tubular epithelial cells. Ion transport can be modulated pharmacologically by the actions of diuretic drugs to regulate urinary volume and composition. Pharmacologic reduction of ion reabsorption leads to reduction of the osmotic driving force that favors water reabsorption in the water-permeable segments of the nephron. Diuretics target sodium reabsorption along four segments of the nephron: the proximal tubule, medullary thick ascending limb, distal convoluted tubule, and collecting duct. Most diuretics need not be administered at doses required to achieve high systemic drug concentrations. The kidney concentrates and secretes these drugs into the tubule lumen, allowing diuretics to reach higher concentrations in the tubule lumen than in the blood.

## Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors, exemplified by **acetazolamide,** inhibit sodium reabsorption by noncompetitively and reversibly inhibiting proximal tubular cytoplasmic carbonic anhydrase II and lumenal carbonic anhydrase IV (Fig. 19-6). Inhibition of carbonic anhydrase activity leads to increased delivery of sodium bicarbonate to more distal segments of the nephron. Much of this sodium bicarbonate is initially excreted, resulting in a decrease of plasma volume (diuresis). However, over several days, the drug's diuretic effect is diminished by compensatory upregulation of $NaHCO_3$ and NaCl absorption across more distal nephron segments (by incompletely understood mechanisms).

Use of carbonic anhydrase inhibitors is often associated with mild-to-moderate metabolic acidosis, arising not only from inhibition of proximal tubular $H^+$ secretion, but also from inhibition of carbonic anhydrase in acid-secreting intercalated cells of the collecting duct. The urine is also alkalinized as a result of carbonic anhydrase inhibition; this increases the urinary excretion of organic acid anions, including drugs such as aspirin.

The clinical use of carbonic anhydrase inhibitors is primarily restricted to several carbonic anhydrase-dependent conditions (see below). In CHF where use of loop agents can cause metabolic alkalosis, carbonic anhydrase inhibitors are occasionally used to restore acid-base balance.

Carbonic anhydrase inhibitors also have ophthalmologic applications. The ciliary process epithelium of the anterior chamber of the eye secretes sodium chloride into the aqueous humor. This NaCl secretion requires carbonic anhydrase activity, because a portion of the basolateral $Cl^-$ uptake by the ciliary epithelium requires coupled $Cl^-$-$HCO_3^-$ and $Na^+$-$H^+$ exchange processes, as well as $Na^+$-$HCO_3^-$ symport. The basolateral membrane $Na^+$-$K^+$-$2Cl^-$ cotransporter NKCC1 mediates most remaining $Cl^-$ uptake by ciliary epithelial cells. **Glaucoma** is characterized by increased pressure in the anterior chamber of the eye. This is usually attributed to partially obstructed outflow of aqueous humor, but in some cases overproduction of aqueous humor may also contribute. Inhibition of carbonic anhydrase in the ciliary process reduces secretion of aqueous humor and may relieve the intraocular pressure of glaucoma. Topical, lipophilic carbonic anhydrase inhibitors are often used in concert with antagonists of β-adrenergic receptors (see Chapter 8, Adrenergic Pharmacology) in the treatment of glaucoma.

Ascent to altitudes greater than 3,000 m above sea level predisposes several body organs, including the brain, to edema and ionic disequilibria. Symptoms of **acute mountain sickness** can include nausea, headache, dizziness, insomnia, pulmonary edema, and confusion. The choroid plexus of the cerebral ventricles secretes both chloride and bicarbonate in cerebrospinal fluid. Inhibition of carbonic anhydrase can be used prophylactically against acute mountain sickness. The still-controversial mechanism(s) include effects on the choroid plexus and ependyma, on the respiratory control centers of the brain, and on the blood–brain barrier.

Part of the treatment of hyperuricemia or **gout** (see Chapter 46, Integrative Inflammation Pharmacology: Gout) is alkalinization of the urine to increase urinary solubility of uric acid. This alkalinization can be achieved by oral bicarbonate supplementation and therapy with carbonic anhydrase inhibitors to reduce renal bicarbonate reabsorption. Maintenance of alkaline urine can prevent uric acid nephrolithiasis (kidney stones) and uric acid nephropathy.

## Osmotic Diuresis

Osmotic diuretics such as **mannitol** are small molecules that are filtered at the glomerulus but not subsequently reabsorbed in the nephron. Thus, they constitute an intralumenal osmotic force limiting reabsorption of water across water-permeable nephron segments. The effect of osmotic agents is greatest in the proximal tubule, where most iso-osmotic reabsorption of water takes place. The consequent increased urine volume can also promote natriuresis. Without replacement of water losses, water loss in excess of sodium excretion can lead to hypernatremia. Mannitol is used primarily for rapid (emergency) reduction of **intracranial pressure** in the setting of head trauma, brain hemorrhage, or a symptomatic cerebral mass. Intracranial pressure can be reduced, at least transiently, by reduction of cerebral intravascular volume.

Osmotic diuresis can also occur as a result of pathologic states. Two common examples of this phenomenon are hyperglycemia and the use of radiocontrast dyes. In diabetic hyperglycemia, the filtered glucose load exceeds the reabsorptive capacity of the proximal tubule for glucose. As a result, significant quantities of glucose remain in the nephron lumen and act as an osmotic agent to increase fluid retention at the tubular lumen, thereby decreasing fluid reabsorption. Radiocontrast agents, which are used in radiologic imaging studies, are filtered at the glomerulus but not reabsorbed by the tubular epithelium. Thus, the dyes constitute an osmotic load and can produce osmotic diuresis. In patients with borderline cardiovascular status, the consequent reduction in intravascular volume can lead to renal and/or cardiac insufficiency secondary to reduced organ perfusion.

## Loop Diuretics

Diuretics acting at the thick ascending limb (TAL), the so-called loop diuretics, reversibly and competitively inhibit the $Na^+$-$K^+$-$2Cl^-$ cotransporter NKCC2 of the thick ascending limb of the loop of Henle on its lumenal surface (Fig. 19-7). Inhibition of transcellular NaCl transport by TAL cells also reduces or abolishes the lumen-positive transepithelial potential difference across the intact TAL. Consequently, paracellular reabsorption of divalent cations, particularly calcium and magnesium, is also inhibited. This results in increased delivery of lumenal calcium and magnesium to downstream reabsorptive sites in the distal convoluted tubule and can lead to increased excretion of calcium and magnesium. Hypomagnesemia can, therefore, be a problem in some patients who require prolonged administration of loop agents. Furthermore, increased downstream delivery of sodium increases the $Na^+$ load presented to principal cells of the collecting duct, which results in increased absorption of $Na^-$ and increased secretion of $K^+$ and protons. This predisposes to hypokalemia and metabolic alkalosis, a clinical picture often described as **volume contraction alkalosis.**

The prototypical loop diuretic is **furosemide.** Other drugs in this class include **bumetanide, torsemide,** and **ethacrynic acid.** All these agents are generally well tolerated. Apart from their effects on renal electrolyte handling, loop diuretics are associated with dose-related **ototoxicity,** presumably because of alteration in endolymph electrolyte handling. For this reason, loop diuretics should not be co-administered with aminoglycosides (see Chapter 33, Pharmacology of Transcription and Translation). The major differences among the loop diuretics are potency and incidence of allergies. Bumetanide is approximately forty times more potent than the other loop diuretics. Furosemide, bumetanide, and torsemide are all **sulfonamide derivatives,** while ethacrynic acid is not. Therefore, ethacrynic acid is a therapeutic option for patients who are allergic to sulfonamide agents.

The high sodium reabsorption capacity of the TAL makes loop diuretics a front-line therapy for acute relief of pulmonary and peripheral edema in the context of **heart failure.** Reduction of intravascular volume with loop diuretics can decrease filling pressures below the thresholds for pulmonary and peripheral edema. This was the rationale for the intravenous furosemide used to treat Mr. Reilly's pulmonary edema and peripheral edema in the introductory case. **Edematous states** secondary to the diminished oncotic pressure of hypoalbuminemia, as in nephrotic proteinuria or liver disease (which results in reduced plasma protein synthesis), can also be treated with low-dose loop diuretics.

Loop agents may also be used therapeutically to increase calcium diuresis in hypercalcemic states, such as hyperparathyroidism or malignancy-associated hypercalcemia caused by tumor secretion of parathyroid hormone-related protein or other calciotropic hormones. Intravenous loop diuretic therapy can thus provide acute relief for **hypercalcemia** (see Chapter 29, Pharmacology of Bone Mineral Homeostasis). Alternatively, loop agents are used to counteract **hyperkalemia** caused by potassium-retaining side effects of other drugs or by renal insufficiency and impaired urinary $K^+$ excretion in the context of normal or elevated dietary $K^+$ intake.

In **acute renal failure,** the increase in urine flow brought about by loop diuretics can facilitate clinical management of fluid balance during decreased glomerular filtration. However, there is no evidence to support the oft-repeated claim that increased urine output itself intrinsically enhances renal tubular epithelial cell recovery from the ischemic or toxic precipitants of acute renal failure.

## Thiazides

Thiazide diuretics inhibit sodium chloride reabsorption in the distal convoluted tubule (Fig. 19-8). These agents act from the apical side as competitive antagonists of the NCC1 $Na^+$-$Cl^-$ cotransporter of the lumenal membrane of distal convoluted tubule cells. The modest natriuresis produced by thiazides stems from the fact that 90% of sodium reabsorption occurs upstream of their site of action in the nephron; nonetheless, thiazides do cause a modest reduction in intravascular volume. The decrease in intravascular volume, possibly combined with a poorly understood direct vasodilatory effect, decreases systemic blood pressure and afterload.

The distal tubule is also a site of parathyroid hormone-regulated reabsorption of calcium via voltage-independent ECaC/CaT channels and perhaps additional yet-to-be-identified voltage-dependent $Ca^{2+}$ channels. Thiazides promote increased transcellular calcium reabsorption in the distal convoluted tubule. Thiazides have been used to decrease urinary $Ca^{2+}$ wasting in **osteoporosis** (although this is no longer common practice in the absence of hypercalciuria) and to diminish hypercalciuria in those at risk for **nephrolithiasis.** The mechanism by which inhibition of NaCl uptake enhances apical $Ca^{2+}$ entry remains a matter of speculation. One hypothesis is that decreased intracellular $Cl^-$ concentration leads to cellular hyperpolarization mediated by open basolateral $Cl^-$ channels, thus increasing the electrical driving force favoring apical $Ca^{2+}$ entry.

**Hydrochlorothiazide** is the prototypical thiazide diuretic. In addition to its effects on renal electrolyte handling, hydrochlorothiazide decreases glucose tolerance, and may unmask diabetes in patients at risk for impaired glucose metabolism. The mechanism of this effect is unknown, but may be attributable to drug-induced impairment of insulin secretion and/or peripheral insulin sensitivity. Thiazide diuretics should not be administered concurrently with antiarrhythmic agents that prolong the QT interval (e.g., quinidine, sotalol), as co-administration of these drugs predisposes patients to *torsades de pointes* (polymorphic ventricular tachycardia, see Chapter 17, Pharmacology of Cardiac Rhythm). The mechanism of this interaction is unknown, but may be related to thiazide-induced hypokalemia, which increases cardiac arrhythmogenic activity (see Chapter 17).

Thiazide diuretics are first-line agents for treatment of **hypertension** (see Chapter 23) because they have been shown to reduce significantly both cardiovascular-related and total mortality in numerous randomized clinical trials. In addition, thiazide diuretics are often used together with loop agents for their synergistic diuretic effects in **congestive heart failure.** This synergism arises because the increased $Na^+$ load, delivered from the loop diuretic-blocked TAL to the thiazide diuretic-blocked DCT, must now proceed further to the collecting duct, which has only a limited ability to up-regulate compensatory $Na^+$ reabsorption. However, care must be taken in setting thiazide doses, for as with loop diuretics, thiazides can increase potassium and proton secretion by increasing sodium presentation to the collecting duct, thus leading to hypokalemic metabolic alkalosis.

Patients with impaired secretion of vasopressin by the posterior pituitary gland, or with impaired signaling by the $V_2$ vasopressin receptor of the collecting duct principal cells, fail to reabsorb water in the terminal nephron. These patients generate large volumes of hypotonic urine. Central diabetes insipidus (defective pituitary secretion of vasopressin) can be treated with the exogenous vasopressin agonist **desmopressin** (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). Patients with nephrogenic diabetes insipidus do not respond to desmopressin; paradoxically, however, thiazide diuretics can produce a modest decrease in urine flow in this setting. By reducing intravascular volume and decreasing glomerular filtration rate, the volume of tubular fluid delivered to the water reabsorption-incompetent collecting duct is reduced, leading in turn to a decreased urine volume.

## Collecting Duct (Potassium-Sparing) Diuretics

In contrast to all other diuretic classes, potassium-sparing diuretics increase nephron reabsorption of potassium. Agents in this class interrupt collecting duct principal cell $Na^+$ reabsorption by one of two mechanisms. Agents such as spironolactone inhibit the biosynthesis of new $Na^+$ channels in the principal cells, while agents such as amiloride and triamterene inhibit the activity of $Na^+$ channels in the lumenal membranes of these cells (Fig. 19-9).

The epithelial sodium channel (ENaC) of collecting duct principal cells is comprised of $\alpha$, $\beta$, and $\gamma$ subunits assembled in a complex with a (still-debated) stoichiometry of $\alpha_2\beta\gamma$. Control of sodium channel expression is regulated primarily by aldosterone, which is secreted by the adrenal cortical zona glomerulosa under regulation of angiotensin II and plasma potassium. Circulating aldosterone diffuses into collecting duct principal cells and binds to an intracellular mineralocorticoid receptor. Activation of the mineralocorticoid receptor increases transcription of mRNAs that encode subunits of ENaC in the apical membrane and also $\alpha$ and $\beta$ subunits of $Na^+/K^+$-ATPase in the basolateral membrane. The increased ENaC expression increases $Na^+$ flux across the lumenal membrane, while increased $Na^+/K^+$-ATPase activity increases $Na^+$ flux from the cytoplasm across the basolateral membrane into the interstitium. These two actions of aldosterone increase $Na^+$ reabsorption and hence increase intravascular volume.

**Spironolactone** inhibits aldosterone action by binding to and preventing nuclear translocation of the mineralocorticoid receptor. **Amiloride** and **triamterene** are competitive inhibitors of the principal cell apical membrane ENaC $Na^+$ channel. Both types of potassium-sparing diuretics can cause **hyperkalemic metabolic acidosis,** because inhibition of electrogenic $Na^+$ uptake by either mechanism decreases the normal transepithelial lumen-negative potential and, thus, decreases the driving force for potassium secretion from collecting duct cells, predisposing to $K^+$ retention and hyperkalemia. Decreased $Na^+$ uptake through ENaC may also diminish $H^+$ secretion. Spironolactone inhibits the androgen receptor; this cross-reactivity can cause impotence and gynecomastia in men.

Potassium-sparing diuretics are mild diuretics when used in isolation because the collecting duct reabsorbs only 1–5% of filtered sodium. However, they can be used to potentiate the action of more proximally acting diuretics, including the loop diuretics. Potassium-sparing diuretics are occasionally used in combination with the potassium-wasting thiazides when thiazide use has led to hypokalemia. Amiloride and triamterene are the drugs of choice for treatment of Liddle syndrome, a rare Mendelian form of hypertension resulting from gain-of-function mutations of the β or γ subunits of the ENaC $Na^+$ channel.

Potassium-sparing diuretics are also used clinically for hypokalemic, alkalotic states secondary to mineralocorticoid excess. Mineralocorticoid excess can accompany CHF, hepatic failure, and other disease processes associated with diminished aldosterone metabolism. The mild diuretic actions of spironolactone minimize the risk of cardiovascular compromise from excessively rapid or extensive diuresis when diminished oncotic pressure impairs the mobilization of extravascular fluid into the vasculature. Therefore, spironolactone is the diuretic of choice for ascites and edema associated with impaired plasma protein biosynthesis secondary to hepatic failure.

Studies have also suggested that spironolactone and other inhibitors of mineralocorticoid action retard the development of heart failure. The mechanism of this effect is unknown, but may be related to inhibition of cardiac fibrosis resulting from a paracrine aldosterone-signaling pathway. Recently, the selective aldosterone receptor antagonist **eplerenone** was approved for use as an antihypertensive agent. This drug has also been shown to reduce mortality in patients with CHF and in patients with significant cardiac dysfunction (ejection fraction less than 40%) after myocardial infarction.

## Conclusion and Future Directions

This chapter has reviewed the physiology and pathophysiology of extracellular volume regulation. Control of intravascular volume maintains adequate perfusion pressure to organs and ensures that the kidney is able to filter waste products from the plasma. Regulation of extracellular volume is accomplished by integrated neurohormonal mechanisms that respond to changes in arterial and atrial wall stress. These hormones act to alter numerous steps in renal $Na^+$ handling and, therefore, maintain a homeostatic balance between $Na^+$ input and excretion. Edema can develop when the capillary hydrostatic pressure gradient favoring fluid filtration exceeds the opposing oncotic forces, which favor fluid entry into the intravascular space. Pharmacologic treatment of dysregulated extracellular volume involves modification of neurohormonal signaling or direct inhibition of renal $Na^+$ reabsorption. ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II; drugs in this class have important vasodilatory actions. Angiotensin receptor antagonists may also prove useful in interruption of the angiotensin–aldosterone axis. Natriuretic peptide has recently been approved for use in the treatment of decompensated heart failure.

Diuretics are agents that alter nephron $Na^+$ reabsorption and, secondarily, alter the reabsorption and secretion of other ions. Essential to understanding diuretic mechanisms is an appreciation of the functional organization of the nephron. With the exception of osmotic diuretics, which increase urinary flow by osmotic retention of water throughout the nephron, a specific class of diuretic drugs targets each of the four segments of the nephron. Such carbonic anhydrase inhibitors as acetazolamide decrease sodium bicarbonate reabsorption in the proximal tubule; such loop agents such as furosemide decrease sodium chloride reabsorption by the apical $Na^+$-$K^+$-$2Cl^-$ pump in the thick ascending loop of Henle; thiazides, such as hydrochlorothiazide, inhibit the apical $Na^+$-$Cl^-$ cotransporter in the distal convoluted tubule; and such potassium-sparing diuretics as spironolactone and amiloride inhibit, respectively, the aldosterone receptor and the ENaC apical $Na^+$ channel in the collecting duct. The most important use of diuretics is for the treatment of hypertension; the second most important use is to treat edema of any cause.

Future developments in the pharmacology of extracellular volume regulation will likely focus on interrupting the hormonal pathways implicated in the disruption of volume homeostasis. This research will focus on new drugs that interrupt the renin-angiotensin–aldosterone axis, including renin inhibitors, neutral endopeptidase inhibitors, additional $AT_1$ receptor inhibitors, and agents that are selective for the aldosterone receptor. Recombinant natriuretic peptides may play an increasingly important role in the management of decompensated heart failure. Drugs interrupting these hormonal pathways have the potential to modify the pathophysiology of these disease processes, and therefore interrupt disease progression while concomitantly regulating renal $Na^+$ reabsorption.

## Suggested Readings

Koeppen BM, Stanton BA, Koeppen BH. Renal Physiology. 3rd ed. Mosby: 2001. (*Concise monograph on renal physiology.*)

Levin ER, Gardner DG, Sampson WK. 1998. Mechanisms of disease: Natriuretic peptides. N Engl J Med 339: 321–328. (*Overview of natriuretic peptide physiology and the role of natriuretic peptides in heart failure.*)

Okusa MD and Ellison DH. Physiology and pathophysiology of diuretic action. In: Seldin DW, Giebisch G, Eds. The Kidney: Physiology and Pathophysiology, 3rd edition. Philadelphia: Lippincott Williams & Wilkins 2000. (*Discusses the physiology and pathophysiology of diuretic action.*)

Opie LH, Yusuf S, Poole-Wilson PA, and Pfeffer M. Angiotensin converting enzyme (ACE) inhibitors, angiotensin-II receptor blockers (ARBs), and aldosterone antagonism. In Opie L, Gersh BJ, Eds. Drugs for the Heart, 5th Ed. Philadelphia: WB Saunders, 2001. (*Clinical information on angiotensin physiology and ACE inhibitors.*)

**Drug Summary Table** Pharmacology of Volume Regulation

| Drug | Clinical Use | Side Effects/Toxicity | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME (ACE)**<br>*Mechanism—Inhibit conversion of ATI to ATII; also increase bradykinin by ↓ degradation* | | | | |
| **Captopril** | All ACE inhibitors can be used in:<br>Hypertension (especially in diabetes)<br>CHF<br>Myocardial infarction | All ACE inhibitors can cause:<br>Cough<br>Angioedema<br>Hypotension<br>Acute renal failure (bilateral renal artery stenosis)<br>Hyperkalemia<br>Rash | Hyperkalemia can occur with $K^+$-sparing diuretics<br>All ACE inhibitors are contraindicated in:<br>Pregnancy<br>Renal failure<br>Bilateral renal artery stenosis | First ACE inhibitor. Active agent, but also metabolized to active metabolites |
| **Enalapril**<br>**Benazepril**<br>**Fosinopril**<br>**Trandolapril**<br>**Quinapril**<br>**Ramipril**<br>**Moexipril**<br>**Perindopril** | | | | Ester prodrugs of ACE inhibitor; converted by liver to active metabolite |
| **Lisinopril** | | | | Excreted unchanged by kidney |
| **INHIBITORS OF ANGIOTENSIN RECEPTORS**<br>*Mechanism—Competitive antagonists of $AT_1$ receptor* | | | | |
| **Candesartan**<br>**Losartan**<br>**Irbesartan**<br>**Telmisartan**<br>**Valsartan** | All $AT_1$-antagonists can be used in hypertension | | All $AT_1$-antagonists are contraindicated in:<br>Pregnancy<br>Bilateral renal artery stenosis | Common alternatives to ACE inhibitor because do not induce cough |
| **NATRIURETIC PEPTIDE**<br>*Mechanism—Multiple, including vascular smooth muscle relaxation and antagonism of ADH* | | | | |
| **Nesiritide** | Decompensated CHF | Hypotension | Caution when used in combination with ACE inhibitors | Recombinant, human sequence B-type natriuretic peptide |
| **CARBONIC ANHYDRASE INHIBITORS**<br>*Mechanism—Noncompetitively inhibit carbonic anhydrase II and IV (proximal tubule; apical membrane and cytoplasm)* | | | | |
| **Acetazolamide** | CHF, glaucoma, gout<br>Mountain sickness | Metabolic acidosis | | |

*(Continued)*

Drug Summary Table

## Pharmacology of Volume Regulation *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **OSMOTIC DIURETICS**<br>*Mechanism—Act as an osmole, opposing water reabsorption along the nephron* | | | | |
| **Mannitol** | ↑ Intracranial pressure<br>↑ Intraocular pressure | Hypernatremia | | Diabetic glycosuria and radiocontrast agents also act as osmotic diuretics<br>Also used rarely in the treatment of compartment syndrome (increased fascial compartment pressures) |
| **LOOP DIURETICS**<br>*Mechanism—Competitively inhibit $Na^+$-$K^+$-$2Cl^-$ cotransporter (thick ascending limb of Henle; apical membrane)* | | | | |
| **Furosemide**<br>**Bumetanide**<br>**Torsemide**<br>**Ethacrynic acid** | All loop diuretics can be used in:<br>CHF, acute renal failure<br>Edematous states (see Notes)<br>Hypercalcemia<br>Hyperkalemia | All loop diuretics can cause:<br>Volume contraction<br>Alkalosis<br>Ototoxicity<br>Hypokalemia<br>Hyperuricemia | All loop diuretics can interact with aminoglycosides (increased ototoxicity and nephrotoxicity) | Loop diuretics are used for nephritic or nephrotic edema; used for cirrhotic ascites (only at very low doses)<br>Ethacrynic acid is not a sulfonamide derivative |
| **THIAZIDE DIURETICS**<br>*Mechanism—Competitively inhibit $Na^+$-$Cl^-$ co-transporter (distal convoluted tubule; apical membrane)* | | | | |
| **Hydrochlorothiazide**<br>**Hydroflumethiazide**<br>**Chlorthalidone**<br>**Bendroflumethiazide**<br>**Polythiazide**<br>**Metolazone**<br>**Indapamide** | All thiazide diuretics can be used in:<br>CHF and hypertension<br>Osteoporosis (rare)<br>Nephrogenic diabetes insipidus | All thiazide diuretics can cause:<br>Hypokalemic metabolic alkalosis<br>Impaired glucose tolerance<br>Hyperuricemia | All thiazide diuretics can interact with quinidine (*torsades de pointes*) | Thiazide diuretics are also used in patients predisposed to nephrolithiasis (diminishes calciuria) |
| **POTASSIUM-SPARING DIURETICS**<br>*Mechanism—Decrease collecting duct $Na^+$ reabsorption; see Notes.* | | | | |
| **Spironolactone** | CHF, liver failure | Hyperkalemia<br>Metabolic acidosis<br>Gynecomastia | | Mineralocorticoid receptor antagonist (collecting duct; intracellular)<br>Favored agent in ascites of cirrhosis |
| **Eplerenone** | CHF, post-MI cardiac dysfunction, hypertension | Hyperkalemia<br>Metabolic acidosis | | Selective mineralocorticoid receptor antagonist |
| **Amiloride**<br>**Triamterene** | CHF, Liddle syndrome | Hyperkalemia | | Inhibit $Na^+$ channel by pore block (collecting duct; apical membrane) |

# 20

# Pharmacology of Vascular Tone

Deborah C. Yeh and Thomas Michel

**Introduction**
**Case**
**Physiology of Vascular Smooth Muscle Contraction and Relaxation**
- Vascular Resistance and Capacitance
- Vascular Smooth Muscle Contraction and Relaxation
- Regulation of Vascular Tone
  - *Vascular Endothelium*
  - *Autonomic Nervous System*
  - *Neurohormonal Mechanisms*
  - *Local Mechanisms*

**Pharmacologic Classes and Agents**
- Organic Nitrates and Sodium Nitroprusside
  - *Mechanism of Action*
  - *Pharmacokinetics*
  - *Pharmacologic Tolerance*
  - *Effects of Nitrates in Addition to Vasodilation*
  - *Contraindications*
- Phosphodiesterase Inhibitors
- $Ca^{2+}$ Channel Blockers
  - *Mechanism of Action*
  - *Chemical Classes*
  - *Pharmacokinetics*
  - *Toxicities and Contraindications*
- $K^+$ Channel Openers
- Other Drugs That Modulate Vascular Tone
  - *Hydralazine*
  - *$\alpha_1$-Adrenergic Antagonists*
  - *β-Adrenergic Antagonists*
  - *Renin–Angiotensin System Blockers*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

In combination with cardiac output, **vascular tone** (i.e., the degree of contraction of vascular smooth muscle) determines the adequacy of perfusion of the tissues of the body. The importance of vascular tone is underscored by the wide spectrum of disease states—ranging from angina pectoris to hypertension to Raynaud's phenomenon to migraine headaches—that are associated with dysregulated vascular tone. As the major factors that govern the regulation of blood vessel diameter have become understood at the molecular level, it has become apparent that a complex array of mechanisms is required to maintain proper vascular tone in the face of diverse stimuli. Pharmacologic strategies for intervention in these regulatory pathways have already yielded many successful therapies for disorders of vascular tone, and provide hope that, in the future, even better therapies will be available to manage the multiple types of vascular disorders.

## Case

GF, a 63-year-old man with a history of hypertension, diabetes, and hypercholesterolemia, begins to develop episodes of chest pain on exertion. One week after his first episode, a bout of chest pain occurs while he is mowing the lawn. Twenty minutes after the onset of this pain, GF takes two of his wife's sublingual nitroglycerin tablets. Within a few minutes of taking his wife's nitroglycerin, he feels much better. In fact, GF feels so well that he decides to take one of the sildenafil (Viagra®) pills that a friend had previously offered to him. A few minutes after taking sildenafil, he feels flushed, develops a throbbing headache, and feels his heart racing. Upon standing, GF feels lightheaded and faints. He is taken immediately to the Emergency Department, where he is found to have severe hypotension. He is quickly placed in a supine position with his legs raised, and monitored until he regains consciousness. The physician considers administering an α-adrenergic agonist, such as phenylephrine, but the rapid amelioration of GF's hypotension after he is placed in a supine position suggests that pharmacologic intervention is unnecessary. After GF recovers, his physician discusses with him the dangers of taking medications without a prescription and, specifically, the risk of concurrent administration of organic nitrates and sildenafil.

### QUESTIONS

- **1.** What is the mechanism by which nitroglycerin acts so quickly to relieve chest pain?
- **2.** What are the common side effects of nitroglycerin?

3. How can sildenafil and organic nitrates interact to precipitate severe hypotension?
4. Besides the organic nitrates, what vasodilating agents are commonly used?
5. Are non-nitrate antihypertensives, such as calcium channel blockers, also contraindicated for men taking sildenafil? How can the mechanisms of action of drugs be used to predict possible interactions or lack of interactions?

## PHYSIOLOGY OF VASCULAR SMOOTH MUSCLE CONTRACTION AND RELAXATION

Vascular tone is a key regulator of tissue perfusion, which determines whether tissues receive sufficient $O_2$ and nutrients to meet their demands. The delicate balance between $O_2$ supply and demand is critical for the function of all tissues, especially for the myocardium. Vascular tone is an important determinant of both myocardial $O_2$ supply and demand. Myocardial $O_2$ supply depends on the tone of the coronary arteries, while myocardial $O_2$ demand depends on the tone of both the systemic arterioles (resistance vessels) and veins (capacitance vessels).

### VASCULAR RESISTANCE AND CAPACITANCE

The tone of the arterial and venous portions of the circulation play important yet distinctive roles in modulating the balance of myocardial $O_2$ demand. The major determinants of myocardial $O_2$ demand are heart rate, contractility, and ventricular wall stress. Wall stress can be expressed as:

$$\sigma = (P \times r)/2h \qquad \text{Equation 20-1}$$

where $\sigma$ is wall stress, $P$ is ventricular pressure, $r$ is ventricular chamber radius, and $h$ is ventricular wall thickness. **Systolic** and **diastolic ventricular wall stresses** are influenced by systemic **arteriolar** and **venous tone**, respectively. **Arteriolar tone** directly controls **systemic vascular resistance** and, thus, arterial blood pressure.

$$\text{MAP} = \text{SVR} \times \text{CO} \qquad \text{Equation 20-2}$$

where MAP is mean arterial pressure, SVR is systemic vascular resistance, and CO is cardiac output. During systole, the intraventricular pressure must exceed the arterial pressure for blood to be ejected. **Afterload**—defined as systolic ventricular wall stress—is equivalent to the resistance that the ventricle must overcome to eject its contents. Assuming that there is no obstruction between the ventricle and the aorta, systolic arterial blood pressure thus approximates systolic ventricular wall stress (i.e., afterload).

Although the *resistance* of the arterial circulation is the most important parameter determined by arteriolar tone, the *capacitance* of the venous circulation is the most important parameter determined by venous tone. **Venous capacitance,** in turn, regulates the volume of blood returning to the heart, which is a major determinant of the end-diastolic volume of the heart. **Preload**—defined as end-diastolic ventricular wall stress—is equivalent to the stretch on the ventricular fibers just before contraction, which is approximated by end-diastolic volume or pressure. Venous tone, thus, determines the end-diastolic ventricular wall stress (i.e., preload). Figure 20-1 and Table 20-1 summarize the dependence of myocardial $O_2$ supply and demand on the tone of the coronary arteries, systemic arterioles, and capacitance veins, and provide a simplified view of how modulation of the tone of these different types of vessels could alter important parameters of cardiovascular physiology. (See Chapter 23, Integrative Cardiovascular Pharmacology, for a diagram of the overall determinants of myocardial $O_2$ supply and demand.)

Myocardial $O_2$ supply and demand must be balanced carefully to ensure adequate tissue perfusion. **Ischemia** occurs when decreased perfusion leads to an $O_2$ deficit. (In contrast, **hypoxia** occurs when there is $O_2$ deprivation despite adequate perfusion.) **Myocardial ischemia** occurs when myocardial $O_2$ supply and demand are imbalanced, such that coronary blood flow cannot fully meet the $O_2$ needs of the heart. Although there are many potential causes of $O_2$ supply:demand imbalance, most cases of myocardial ischemia—especially coronary artery disease—include some aspect of abnormal vascular tone. For further discussion of the pathophysiology of myocardial ischemia and other diseases of abnormal vascular tone, see Chapter 23.

Chest pain, termed **angina pectoris,** is a common, although not always present, symptom of myocardial ischemia. Given GF's risk factors for coronary artery disease (i.e., diabetes, hypertension, hypercholesterolemia, age, and male gender), and his symptoms of exertional chest pain when mowing the lawn, it is likely that his chest pain was a manifestation of angina pectoris. The most common treatment for angina pectoris is **nitroglycerin,** an agent that decreases vascular tone (see below) and, thereby, ameliorates the mismatch between myocardial $O_2$ supply and demand. Indeed, GF did experience some relief of his chest pain upon taking his wife's nitroglycerin. To understand better how nitroglycerin and other modulators of vascular tone act, it is essential to appreciate the molecular mechanisms that regulate vascular smooth muscle contraction and relaxation.

### VASCULAR SMOOTH MUSCLE CONTRACTION AND RELAXATION

Regulators of vascular tone act by influencing the actin–myosin contractile apparatus of vascular smooth muscle. As in other muscle cells, the actin–myosin interaction leads to contraction and is regulated by the intracellular calcium ($Ca^{2+}$) concentration (Fig. 20-2). A steep transmembrane gradient of $Ca^{2+}$ concentration ($[Ca^{2+}]_{\text{extracellular}} = 2\times10^{-3}$ M; $[Ca^{2+}]_{\text{intracellular}} = 10^{-7}$ M) is maintained by the relative impermeability of the plasma membrane to $Ca^{2+}$ ions and by membrane pumps that actively remove $Ca^{2+}$ from the cytoplasm. Stimulation of vascular smooth muscle cells can increase the cytoplasmic $Ca^{2+}$ concentration by two types of mechanisms. First, $Ca^{2+}$ can enter the cell by way of voltage-gated or metabotropic **$Ca^{2+}$-selective channels** in the sarcolemma. Second, increases in cytoplasmic $[Ca^{2+}]$ can be elicited by the release of intracellular $Ca^{2+}$ from the sarcoplasmic reticulum.

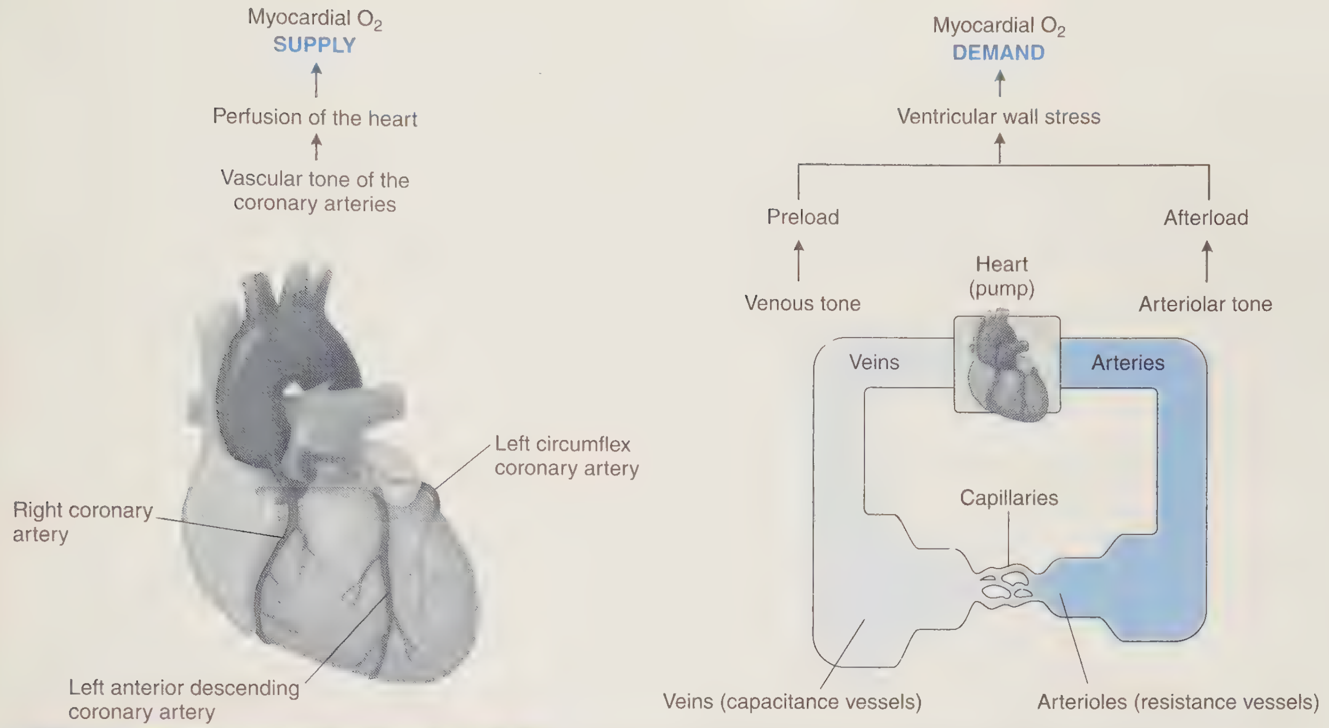

Myocardial $O_2$
SUPPLY
Perfusion of the heart
Vascular tone of the coronary arteries
Left circumflex coronary artery
Right coronary artery
Left anterior descending coronary artery
Myocardial $O_2$
DEMAND
Ventricular wall stress
Preload
Afterload
Venous tone
Heart (pump)
Arteriolar tone
Veins
Arteries
Capillaries
Veins (capacitance vessels)
Arterioles (resistance vessels)

Figure 20-1. Myocardial Oxygen Supply and Demand. Myocardial $O_2$ supply (left panel) is determined by perfusion of the heart, which is determined by the vascular tone of the coronary arteries (among other factors). The major coronary arteries are depicted on the epicardial surface of the heart. Myocardial $O_2$ demand (right panel) is determined by ventricular wall stress, which is a function of both preload (venous tone) and afterload (arteriolar tone). Venous tone determines myocardial $O_2$ demand by altering the amount of blood returning to the heart, which, in turn, determines the end diastolic ventricular wall stress. Arteriolar tone determines myocardial $O_2$ demand by altering systemic vascular resistance (SVR), the pressure against which the heart must contract. Arteriolar tone, therefore, determines systolic ventricular wall stress.

**Vasoconstriction** (i.e., contraction of vascular smooth muscle) is commonly initiated by the opening of **voltage-gated L-type $Ca^{2+}$ channels** in the sarcolemma during plasma membrane depolarization. The open $Ca^{2+}$ channels mediate $Ca^{2+}$ influx, and the $Ca^{2+}$ then binds to cytoplasmic calmodulin (CaM). The $Ca^{2+}$/CaM complex binds to and activates **myosin light chain kinase,** which phosphorylates myosin-II light chains. When the light chain is phosphorylated, the myosin head can interact with an actin filament, leading to smooth muscle contraction (Fig. 20-3, left panel).

**Vasodilation** (i.e., relaxation of vascular smooth muscle) occurs upon dephosphorylation of the myosin light chain.

TABLE 20-1 Relationship Between Vascular Tone and Parameters of Cardiovascular Physiology

| VESSEL TYPE | PARAMETER OF CARDIOVASCULAR PHYSIOLOGY |
|---|---|
| Coronary arteries | Myocardial $O_2$ supply |
| Arterioles | Afterload<br>Myocardial $O_2$ demand<br>Regional myocardial perfusion |
| Capacitance veins | Venous pooling<br>Preload<br>Myocardial $O_2$ demand |

In a simplified model, the effects of pharmacologic agents on cardiovascular physiology can be predicted based on the vessel type on which the agents act. Coronary artery dilators increase myocardial $O_2$ supply. Arteriolar dilators decrease afterload, while venodilators decrease preload; both arteriolar dilators and venodilators decrease myocardial $O_2$ demand.

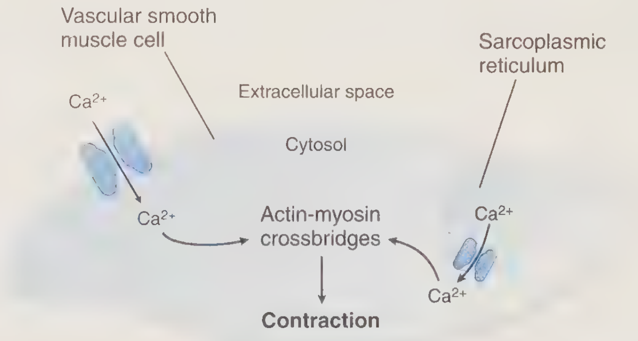

Vascular smooth muscle cell
Sarcoplasmic reticulum
$Ca^{2+}$
Extracellular space
Cytosol
$Ca^{2+}$
Actin-myosin crossbridges
$Ca^{2+}$
$Ca^{2+}$
Contraction

Figure 20-2. Sources of $Ca^{2+}$ for Contraction of Vascular Smooth Muscle Cells. The cytosolic concentration of $Ca^{2+}$ is low ($10^{-7}$ M), while the extracellular and sarcoplasmic reticulum concentrations of $Ca^{2+}$ are high ($2 \times 10^{-3}$ M). $Ca^{2+}$ can enter the vascular smooth muscle cell cytoplasm from the extracellular space or from the sarcoplasmic reticulum via $Ca^{2+}$-selective channels. The increased concentration of $Ca^{2+}$ in the cytosol initiates contraction by promoting the formation of actin–myosin cross-bridges.

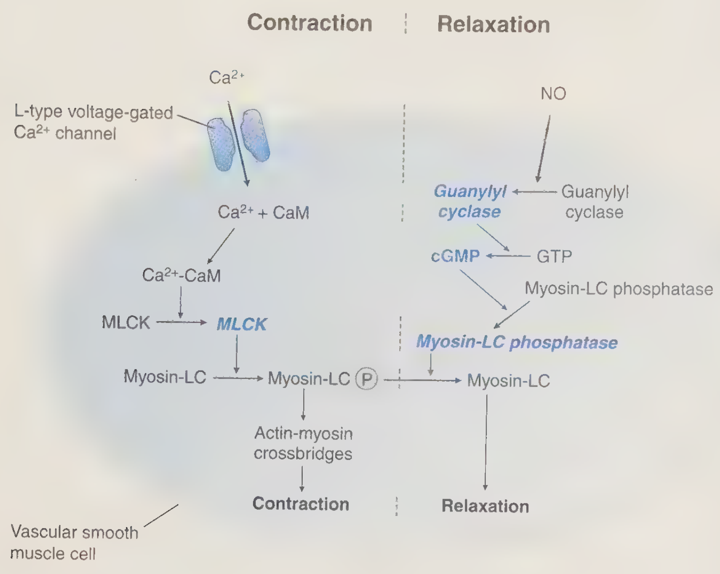


**Figure 20-3. Mechanism of Vascular Smooth Muscle Contraction and Relaxation.** Vascular smooth muscle contraction and relaxation are controlled by the coordinated action of several intracellular signaling mediators. $Ca^{2+}$ entry through L-type voltage-gated $Ca^{2+}$ channels (left panel) is the initial stimulus for contraction. $Ca^{2+}$ entry into the cell activates calmodulin (CaM). The $Ca^{2+}$–CaM complex activates myosin light chain kinase (MLCK) to phosphorylate myosin light chain (Myosin-LC). The phosphorylated myosin-LC interacts with actin to form actin-myosin cross-bridges, a process that initiates vascular smooth muscle cell contraction. Relaxation (right panel) is a coordinated series of steps that act to dephosphorylate (and hence inactivate) myosin-LC. Diffusion of nitric oxide (NO) into the cell activates guanylyl cyclase. The activated guanylyl cyclase catalyzes the conversion of guanosine triphosphate (GTP) to guanosine 3′,5′-cyclic monophosphate (cGMP). cGMP activates myosin-LC phosphatase, which dephosphorylates myosin light chain, preventing actin–myosin cross-bridge formation. As a result, the vascular smooth muscle cell relaxes. The active form of each enzyme is italicized and blue.

Dephosphorylation is potentiated when **guanylyl cyclase** (discussed below) is activated inside the smooth muscle cell. Activated guanylyl cyclase increases the production of **cyclic guanosine 3′5′-monophosphate (cGMP).** The cGMP stimulates **cGMP-dependent protein kinase,** which then activates **myosin light chain phosphatase.** Dephosphorylation of the myosin light chain inhibits the interaction of the myosin head with actin, leading to smooth muscle relaxation (Fig. 20-3, right panel).

## REGULATION OF VASCULAR TONE

Vascular tone is governed by a wide variety of mechanisms. Recent research has highlighted the interactions between vascular endothelial cells and vascular smooth muscle cells in the control of vascular tone. The autonomic nervous system and a number of neurohormonal mediators also control vascular smooth muscle contraction and relaxation. Many of these physiologic mechanisms provide the basis for current drug discovery research.

### Vascular Endothelium

Research in the past two decades has elucidated a number of signaling roles for the vascular endothelium in the control of vascular tone. Endothelial cells elaborate many signaling mediators and alter the expression of many genes in response to diverse stimuli. Two of the most pharmacologically relevant targets, nitric oxide and endothelin, are discussed here.

#### *Nitric Oxide*

The obligatory role of endothelial cells in regulating vascular tone was first described with the observation that acetylcholine causes vasoconstriction when applied directly to de-endothelialized blood vessels, but causes vasodilation when applied to normally endothelialized vessels (Fig. 20-4). It was hypothesized that muscarinic cholinergic stimulation of the endothelium induces the production of a relaxant molecule in the endothelial cell and that this molecule then diffuses to subjacent vascular smooth muscle cells to activate guanylyl cyclase. The putative vasodilatory compound was termed **endothelial-derived relaxing factor,** or **EDRF.**

Before the molecular identity of EDRF was determined to be nitric oxide (NO), nitroglycerin—an **organic nitrate** commonly prescribed for angina pectoris—was known to be metabolized in the body to form NO, and NO was known to cause relaxation of vascular smooth muscle. Based on these two findings, it was later confirmed that the EDRF released from endothelial cells possesses many of the pharmacologic properties of the NO liberated from organic nitrates: both agents stimulate guanylyl cyclase, both are labile, and both are similarly stabilized and degraded by different chemical treatments. In 1986, it was definitively shown that NO is, indeed, the endogenous vasodilator EDRF made by endothelial cells.

Although acetylcholine was the first ligand that was identified to promote endothelial cell synthesis of NO, a number of other mediators have since been described. Shear stress, acetylcholine, histamine, bradykinin, sphingosine 1-phosphate, serotonin, substance P, and ATP can all elicit increased NO synthesis by vascular endothelial cells. NO is synthesized by a family of $Ca^{2+}$/CaM-activated NO synthases. The **endothelial isoform of nitric oxide synthase (eNOS)** is responsible for endothelial cell NO synthesis; this enzyme plays a critical role in controlling vascular tone and platelet aggregation. The importance of NO in regulating vascular tone is underscored by the observation that eNOS-deficient mice are hypertensive.

Recent evidence suggests that NO may effect vasodilation not only by activating guanylyl cyclase, but also by activating $Ca^{2+}$-dependent $K^+$ channels in vascular smooth muscle cells (Fig. 20-4). NO seems to activate these $K^+$ channels directly via a guanylyl cyclase-independent mechanism, leading to hyperpolarization of the cells and, subsequently, to vasodilation. (See below for a further explanation of how opening $K^+$ channels leads to hyperpolarization and vasodilation.)

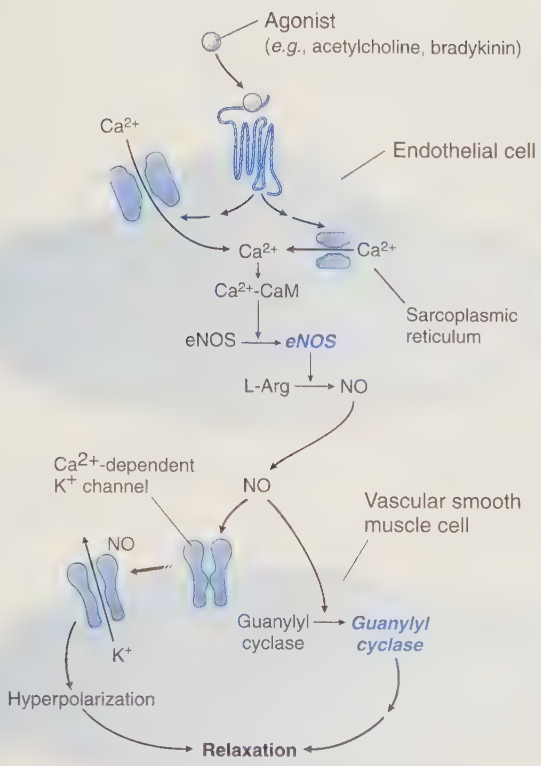

Agonist
(*e.g.*, acetylcholine, bradykinin)
$Ca^{2+}$
Endothelial cell
$Ca^{2+}$
$Ca^{2+}$
$Ca^{2+}$-CaM
Sarcoplasmic reticulum
eNOS
*eNOS*
L-Arg
NO
$Ca^{2+}$-dependent $K^+$ channel
NO
Vascular smooth muscle cell
NO
Guanylyl cyclase
*Guanylyl cyclase*
$K^+$
Hyperpolarization
Relaxation

**Figure 20-4. Endothelial Regulation of Nitric Oxide-Mediated Vascular Smooth Muscle Relaxation.** Endothelial cell production of nitric oxide (NO) controls the extent of vascular smooth muscle relaxation. Production of NO is stimulated by agonists such as acetylcholine or bradykinin. Stimulation of receptors by these agonists activates $Ca^{2+}$ second messenger systems, and promotes direct entry of $Ca^{2+}$ into the cytosol. The increased cytosolic $Ca^{2+}$ activates a $Ca^{2+}$-calmodulin complex that activates endothelial nitric oxide synthase (eNOS), an enzyme that catalyzes the formation of NO from L-arginine (L-Arg, an amino acid). NO diffuses from the endothelial cell into subjacent vascular smooth muscle cells, where it activates guanylyl cyclase, promoting smooth muscle cell relaxation (see Fig. 20-3). NO can also directly activate $Ca^{2+}$-dependent $K^+$ channels. This parallel signaling pathway contributes to relaxation by hyperpolarizing the smooth muscle cell. The active form of each enzyme is italicized and blue.

### *Endothelin*

**Endothelin** is a 21-amino acid vasoconstrictor peptide. It is the most potent endogenous vasoconstrictor yet discovered. Endothelin can be considered a functional "mirror-image" of NO, in that it is a potent endothelially derived vasoconstrictor, while NO is a potent endothelially derived vasodilator. In addition to its effects on the vasculature, endothelin has positive inotropic and chronotropic actions on the heart and contributes to remodeling within the cardiovascular system. Endothelin also plays an important role in the lungs, kidneys, and brain. Three isoforms of endothelin—**ET-1, ET-2,** and **ET-3**—have been identified. ET-1—the isoform mainly involved in cardiovascular actions—is produced by endothelial cells (and vascular smooth muscle cells under inflammatory conditions) and seems to act locally in a paracrine or autocrine fashion. The local ET-1 concentration within the vascular wall is more than 100 times greater than that in the circulation, because ET-1 is secreted chiefly on the basal side of the endothelial cells (Fig. 20-5).

Endothelin precursors are proteolytically processed in two steps to generate the mature active peptides. First, **preproendothelin** is cleaved into **big endothelin.** Second, big endothelin is cleaved by **endothelin-converting enzyme** into endothelin. There are two endothelin receptor subtypes, $ET_A$ and $ET_B$. Both $ET_A$ and $ET_B$ are G protein-coupled receptors whose effectors likely involve phospholipase C-modulated pathways. ET-1 binds to $ET_A$ receptors on vascular smooth muscle cells as well as $ET_B$ receptors on both endothelial cells and vascular smooth muscle cells. $ET_A$ receptors on vascular smooth muscle cells mediate vasoconstriction. $ET_B$ receptors are located predominantly on vascular endothelial cells, where they mediate the release of prostacyclin and NO and, hence, vasodilation. $ET_B$

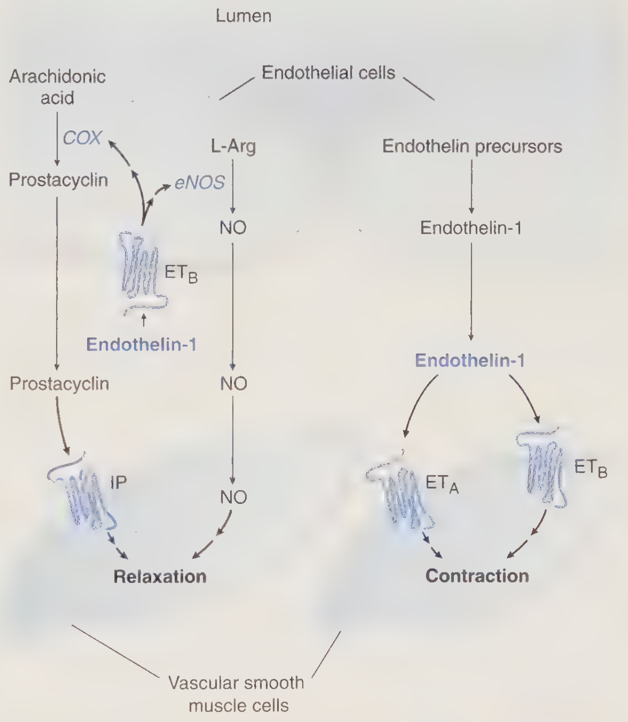

Lumen
Arachidonic acid
Endothelial cells
*COX*
L-Arg
Endothelin precursors
Prostacyclin
*eNOS*
NO
Endothelin-1
$ET_B$
Endothelin-1
Endothelin-1
Prostacyclin
NO
IP
NO
$ET_A$
$ET_B$
Relaxation
Contraction
Vascular smooth muscle cells

**Figure 20-5. Effects of Endothelin on the Blood Vessel Wall.** Endothelin mediates both contraction and relaxation of vascular smooth muscle cells. Endothelin precursors in endothelial cells are processed to endothelin-1. Endothelin-1 is secreted on the basal side of the endothelial cell, where it interacts with $ET_A$ and $ET_B$ receptors present on vascular smooth muscle cells. Activation of these receptors stimulates contraction by incompletely understood mechanisms. $ET_B$ receptors are also expressed on endothelial cells. Endothelial cell $ET_B$ activation stimulates cyclooxygenase (COX), which catalyzes the formation of prostacyclin from arachidonic acid. Prostacyclin diffuses from the endothelial cell to the vascular smooth muscle cell membrane, where it binds to and activates the isoprostanoid (IP) receptor. $ET_B$ activation also stimulates endothelial nitric oxide synthase (eNOS), which catalyzes the formation of NO from arginine (L-Arg). Both prostacyclin and NO stimulate vascular smooth muscle cell relaxation.

receptors are also found on vascular smooth muscle cells, where they mediate vasoconstriction.

### Autonomic Nervous System

Autonomic innervation is an important determinant of vascular tone. The sympathetic nervous system has much greater influence over vascular tone than does the parasympathetic system. The firing of certain sympathetic postganglionic neurons releases norepinephrine from nerve terminals that end on vascular smooth muscle cells. Activation of $\alpha_1$-adrenergic receptors on vascular smooth muscle cells causes vasoconstriction, whereas activation of $\beta_2$-adrenergic receptors on vascular smooth muscle cells induces vasodilation. The effect of norepinehrine at $\alpha_1$-adrenergic receptors is typically greater than its effect at $\beta_2$-adrenergic receptors, especially in organs that receive decreased blood flow during "fight or flight" responses (i.e., skin and viscera). Thus, the net effect of norepinephrine on these vascular beds is typically vasoconstrictive. Because blood vessels are not innervated by parasympathetic fibers, vascular tone is only minimally influenced by the parasympathetic nervous system.

### Neurohormonal Mechanisms

Many neurohormonal mediators act on vascular smooth muscle cells, endothelial cells, and neurons to regulate vascular tone. For example, circulating catecholamines from the adrenal gland (i.e., epinephrine) can influence vascular tone via $\alpha_1$-adrenergic and $\beta_2$-adrenergic receptors on vascular smooth muscle cells: as noted above, stimulation of $\alpha_1$-adrenergic receptors leads to vasoconstriction, while stimulation of $\beta_2$-adrenergic receptors leads to vasodilation. Other examples of neurohormonal mediators include angiotensin II, which stimulates the angiotensin II receptor subtype 1 ($AT_1$ receptor) to vasoconstrict arterioles and increase intravascular volume; aldosterone, which acts via the mineralocorticoid receptor to increase intravascular volume; natriuretic peptides, which promote renal natriuresis (sodium excretion) in situations of volume overload; and antidiuretic hormone/arginine vasopressin, which stimulates arteriolar $V_1$ receptors to constrict arterioles and activates renal $V_2$ receptors to increase intravascular volume. These agents, which have important roles in volume regulation, are all discussed in greater detail in Chapter 19, Pharmacology of Volume Regulation.

### Local Mechanisms

A panoply of local control mechanisms also modulate vascular tone. Autoregulation is a homeostatic mechanism in which vascular smooth muscle cells respond to increases or decreases in perfusion pressure by vasoconstriction or vasodilation, respectively, to preserve blood flow at a relatively constant level (Flow = Perfusion Pressure / Resistance). Vascular tone, and thus blood flow, is also governed by metabolites—such as $H^+$, $CO_2$, $O_2$, adenosine, lactate, and $K^+$—produced in surrounding tissue. Local mechanisms of vascular tone regulation predominate in the vascular beds of essential organs (i.e., heart, brain, lung, kidney), so that blood flow, and thus $O_2$ supply, can be adjusted quickly to meet the demands of local metabolism in these organs.

## PHARMACOLOGIC CLASSES AND AGENTS

The pharmacologic agents considered in this chapter are all **direct vasodilators,** that is, drugs that act on vascular smooth muscle and/or on the adjacent vascular endothelium to decrease vascular tone. Most direct vasodilators act by reducing the contractility of actin–myosin complexes in vascular smooth muscle cells. There are a number of categories of direct vasodilators (Fig. 20-6). Pharmacologic **donors of NO**—such as the **organic nitrates** and **sodium nitroprusside**—cause vasodilation by activating guanylyl cyclase and, thereby, increasing the dephosphorylation of myosin light chains. **$Ca^{2+}$ channel blockers** cause vasodilation by reducing intracellular $Ca^{2+}$ concentrations. **$K^+$ channel openers** induce vasodilation by opening ATP-sensitive $K^+$ channels that hyperpolarize the cell and, thereby, prevent activation of the voltage-gated $Ca^{2+}$ channels necessary for $Ca^{2+}$ influx and muscle contraction. **$\alpha_1$-Adrenergic antagonists** inhibit the vasoconstrictive action of endogenous epinephrine and norepinephrine. **ACE inhibitors** and **angiotensin II receptor subtype 1 ($AT_1$ receptor) antagonists** inhibit the vasoconstrictive effects of endogenous angiotensin II, either by inhibiting angiotensin II formation (ACE inhibitors) or by blocking angiotensin II action at its cognate receptor ($AT_1$ antagonists). **Endothelin receptor antagonists** block endothelin-mediated vasoconstriction. **$\beta$-Adrenergic agonists** and **cAMP phosphodiesterase inhibitors** increase intracellular cAMP and, thereby, cause vascular smooth muscle relaxation.

## ORGANIC NITRATES AND SODIUM NITROPRUSSIDE

Organic nitrates represent one of the oldest cardiac therapies still in use. Indeed, **glyceryl trinitrate**—more commonly termed **nitroglycerin (NTG)**—was first employed for relief of anginal symptoms over 100 years ago. Indications for the use of organic nitrates now include not only the classic indication, stable angina pectoris, but also unstable angina, acute myocardial infarction, hypertension, and acute and chronic congestive heart failure (see Chapter 23).

### Mechanism of Action

Within the body, organic nitrates are chemically reduced to release NO. As described in detail above, NO is an endogenous signaling molecule that causes vascular smooth muscle relaxation. The various organic nitrates likely give rise to NO by different chemical and biochemical mechanisms, but the details of organic nitrate metabolism remain poorly understood. Both extracellular and intracellular reducing agents (e.g., thiol groups) have been implicated (Fig. 20-7). The release of NO from organic nitrates into vascular tissue may also occur by an enzymatic process; it has been hypothesized that the enzymatic release of organic nitrates allows the "targeting" of their effects to specific vascular tissues. Alternatively, tissue-specific nitrate metabolism may be nonenzymatic (e.g., related to thiol pools). Regardless, it is clear that,

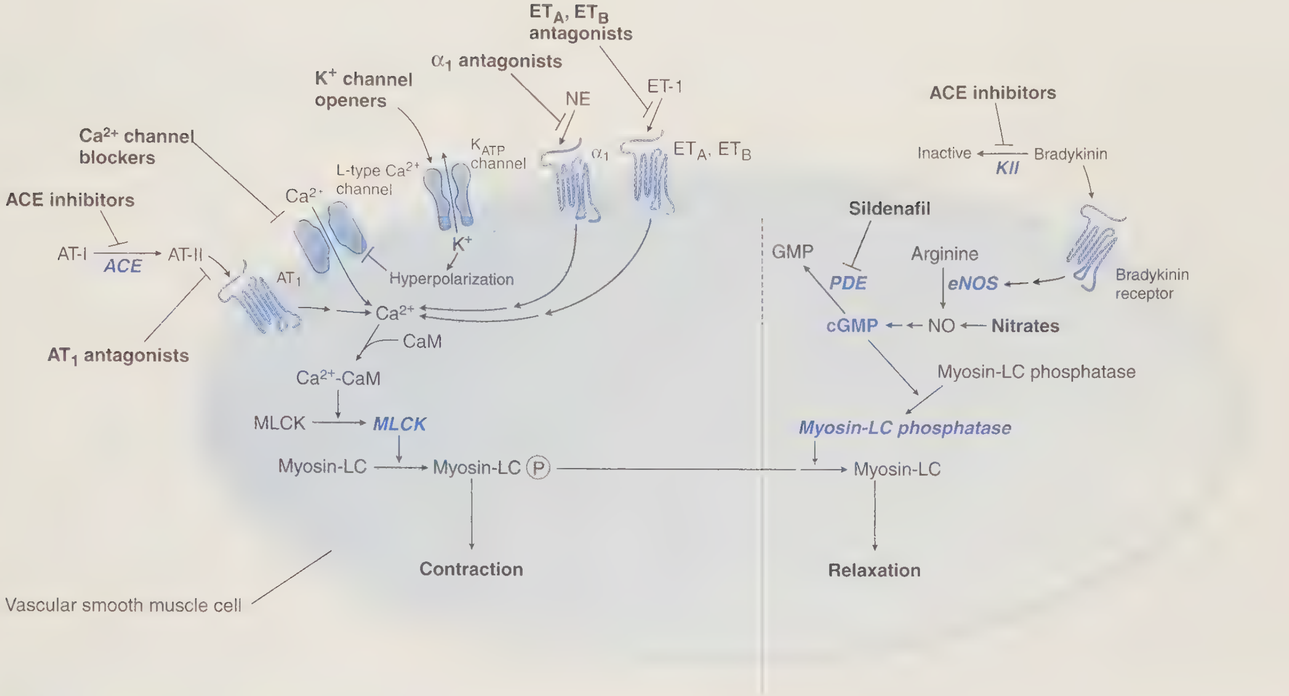


**Figure 20-6. Sites of Action of Vasodilators.** Vasodilators act at several sites in the vascular smooth muscle cell. Left panel: Ca$^{2+}$ channel blockers and K$^+$ channel openers inhibit the entry of Ca$^{2+}$ into vascular smooth muscle cells by decreasing activation of L-type Ca$^{2+}$ channels. ACE inhibitors, AT$_1$ antagonists, $\alpha_1$-antagonists, and endothelin receptor (ET$_A$, ET$_B$) antagonists all decrease intracellular Ca$^{2+}$ signaling. The decreased cytosolic Ca$^{2+}$ results in less vascular smooth muscle cell contraction, and, hence, in relaxation. Right panel: ACE inhibitors inhibit kininase II (KII), leading to increased levels of bradykinin. Nitrates release NO. Sildenafil inhibits phosphodiesterase (PDE). These agents all cause an increase in cGMP, an effect that promotes vascular smooth muscle relaxation. The active form of each enzyme is italicized and blue. $\alpha_1$, $\alpha_1$-Adrenergic receptor. AT-I, angiotensin I. AT-II, angiotensin-II. CaM, calmodulin. eNOS, endothelial nitric oxide synthase. ET-1, endothelin-1. MLCK, myosin light chain kinase. Myosin-LC, myosin light chain.

*although NO can dilate both arteries and veins, venous dilation predominates at therapeutic doses.* NO-induced venodilation increases venous capacitance, leading to a decrease in the return of blood to the right side of the heart and, subsequently, to decreased right ventricular and left ventricular end-diastolic pressure and volume. This decrease in preload reduces myocardial $O_2$ demand. At higher concentrations of organic nitrates, arterial vasodilation may also occur. In the absence of reflex tachycardia, this leads to a decrease in systemic vascular resistance, which leads to a decrease in systolic wall stress (afterload) and a decrease in myocardial $O_2$ demand.

In the coronary circulation, NTG dilates predominantly the large epicardial arteries (Fig. 20-8). NTG has minimal effects on the coronary resistance vessels (i.e., the coronary arterioles). This preferential dilation of the large epicardial arteries over the smaller coronary arterioles prevents the development of the **coronary steal phenomenon,** which is often encountered with such agents as dipyridamole (see Chapter 21) that produce intense dilation of the coronary resistance vessels. The degree to which nitrate-mediated vasodilation of the large epicardial arteries is responsible for the beneficial effects of nitrates in patients with angina remains unclear, however. The chronic myocardial $O_2$ deficit in patients with coronary artery disease may, by an autoregulatory mechanism, cause maximal dilation of the coronary arteries, so that vasodilators can provide no further increase in coronary blood flow. Furthermore, stiffened, calcific atherosclerotic coronary arteries may remain noncompliant, even in the face of coronary artery vasodilators.

Clinically, the administration of doses of organic nitrates sufficient to vasodilate the large epicardial arteries can be dangerous because such doses may also induce excessive peripheral arteriolar vasodilation and refractory hypotension. The excessive decrease in mean arterial pressure can be manifested as dizziness, lightheadedness, and occasionally, overt syncope, and can even lead to myocardial ischemia. Because coronary perfusion depends on the pressure gradient between the aorta and the endocardium during diastole, a decrease in diastolic aortic pressure can lead to an insufficient supply of $O_2$ to the heart. Moreover, systemic hypotension can lead to **reflex tachycardia,** which also decreases myocardial $O_2$ supply by shortening diastole and, thus, myocardial perfusion time. As noted above, reflex tachycardia can also harm the delicate myocardial $O_2$ supply:demand balance by increasing myocardial $O_2$ consumption. Reflex tachycardia is typically observed when baroreceptors in the aortic arch and carotid sinuses sense a decrease in blood pressure. In patients with left heart failure and overt congestive heart failure, however, reflex tachycardia is rare. Thus, nitrates can often be used to decrease pulmonary congestion in patients with congestive heart failure (by effecting venodilation and decreasing end-diastolic pressure), without eliciting significant reflex tachycardia. Several other important side effects of nitrates are also the result of excessive vasodilation; these include flushing,

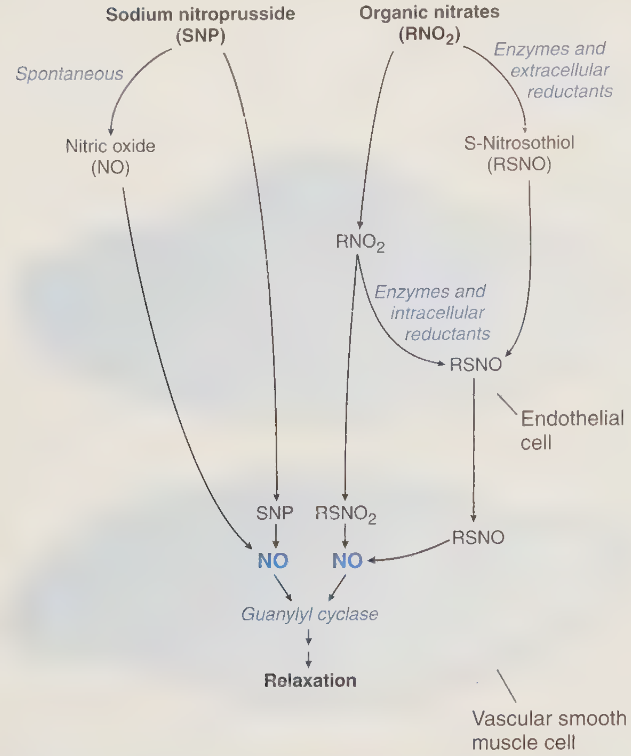

Sodium nitroprusside (SNP)
Organic nitrates ($RNO_2$)
Spontaneous
Enzymes and extracellular reductants
Nitric oxide (NO)
S-Nitrosothiol (RSNO)
$RNO_2$
Enzymes and intracellular reductants
RSNO
Endothelial cell
SNP
$RSNO_2$
RSNO
NO
NO
Guanylyl cyclase
Relaxation
Vascular smooth muscle cell

**Figure 20-7. Biotransformation of Organic Nitrates and Sodium Nitroprusside.** Organic nitrates and sodium nitroprusside increase local levels of NO by different mechanisms. Organic nitrates have the chemical structure $RNO_2$. The nitro group is reduced to form NO in the presence of specific enzymes and extracellular and/or intracellular reductants (e.g., thiols). In comparison, sodium nitroprusside releases NO spontaneously without enzymatic aid. Both agents effect relaxation via the formation of NO. However, the requirement of organic nitrates for specific cellular enzymes and/or reductants may result in tissue selectivity. Because sodium nitroprusside spontaneously converts to NO, it does not dilate particular vascular beds preferentially.

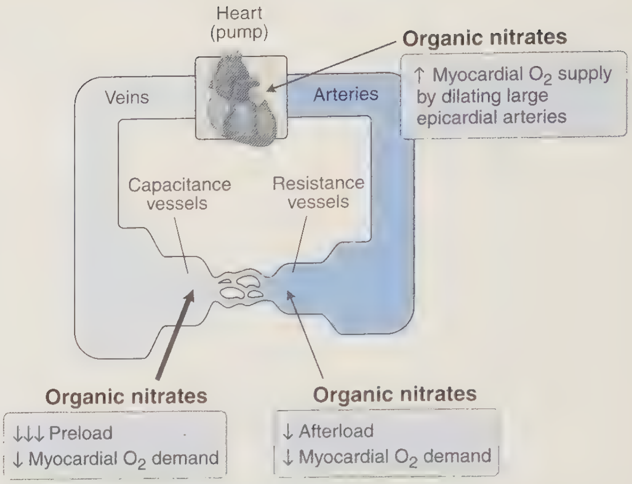

Heart (pump)
Organic nitrates
↑ Myocardial $O_2$ supply by dilating large epicardial arteries
Veins
Arteries
Capacitance vessels
Resistance vessels
Organic nitrates
↓↓↓ Preload
↓ Myocardial $O_2$ demand
Organic nitrates
↓ Afterload
↓ Myocardial $O_2$ demand

**Figure 20-8. Sites of Action of Organic Nitrates.** Organic nitrates exert the majority of their vasodilator action on venous capacitance vessels. This selectivity results in greatly decreased preload, with resulting decreased myocardial $O_2$ demand. Organic nitrates also mildly dilate arteriolar resistance vessels, with resulting decreased afterload and decreased myocardial $O_2$ demand. Myocardial $O_2$ supply is mildly increased by dilation of large epicardial arteries.

caused by vasodilation of cutaneous vascular beds, and headache, caused by vasodilation of cerebral arteries.

Several different preparations of organic nitrates are currently available. The most commonly used organic nitrates include **NTG, isosorbide dinitrate,** and **isosorbide 5-mononitrate** (Fig. 20-9). Although these organic nitrates share a common mechanism of action, they differ in their routes of administration and pharmacokinetics, leading to important differences in their therapeutic utilities in a variety of clinical settings.

**Sodium nitroprusside** is a nitrate compound that consists of a nitroso group, 5 cyanide groups, and iron (Fig. 20-10A). As with the organic nitrates, sodium nitroprusside effects vasodilation by release of NO. Unlike the organic nitrates, however, sodium nitroprusside seems to liberate NO primarily through a nonenzymatic process (Fig. 20-7). As a result of this nonenzymatic conversion to NO, sodium nitroprusside action does not seem to be targeted to specific types of vessels and, consequently, the drug dilates both arteries and veins.

Sodium nitroprusside is used intravenously for potent hemodynamic control in hypertensive emergencies and severe cardiac failure. Because of its rapid onset of action, short duration of action, and high efficacy, sodium nitroprusside must be infused with continuous blood pressure monitoring and careful titration of drug dose to drug effect. Sodium nitroprusside is metabolized rapidly to liberate NO and cyanide (Fig. 20-10B). The cyanide is then converted to thiocyanate in the liver, and the thiocyanate is excreted by the kidneys. Excessive cyanide accumulation can lead to acid-base disturbances, cardiac arrhythmias, and even death. Thiocyanate toxicity can also occur in patients with impaired renal function, causing disorientation, psychosis, muscle spasms, and convulsions.

## Pharmacokinetics

The pharmacokinetics of the different nitrate preparations and formulations provide a basis for the preferential use of specific agents and dosage forms in certain settings. For example, the rapid onset of action of **sublingual nitrate preparations** is desirable for rapid relief of acute anginal attacks, while **longer-acting nitrates** are more valuable for angina prophylaxis in the long-term management of coronary artery disease. Orally administered NTG and isosorbide dinitrate have low bioavailability because organic nitrate reductases in the liver rapidly metabolize these drugs. Both to circumvent the first-pass effect and to attain therapeutic blood levels within minutes, NTG or isosorbide dinitrate can be administered sublingually. **Intravenous administration of NTG** is indicated when continuous titration of drug action is necessary; for example, in the treatment of unstable angina or acute heart failure. **Slow-release transdermal and buccal preparations of NTG** provide therapeutic steady-state levels of NTG that can be useful for angina prevention in patients with stable coronary artery disease.

NTG has a short half-life (approximately 5 minutes), after which it is denitrated into biologically active glyceryl dinitrate metabolites that have longer half-lives (approximately 40 minutes) (Fig. 20-9). Equivalent doses of **isosorbide dinitrate** can be more effective than NTG, because isosorbide dinitrate has

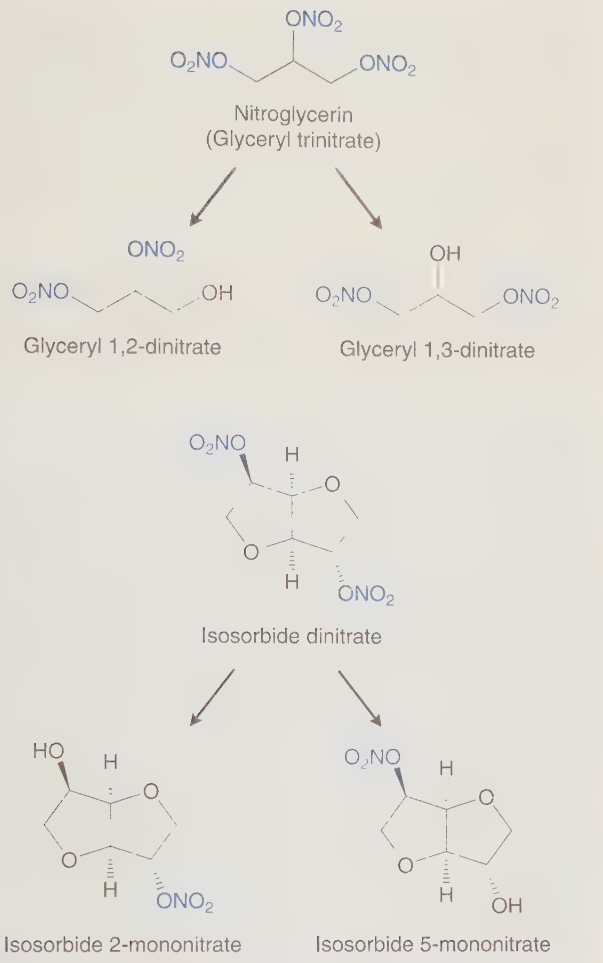


**Figure 20-9. Chemical Structures and Metabolism of Nitroglycerin and Isosorbide Dinitrate.** Nitroglycerin and isosorbide dinitrate are biologically active nitrates that are metabolized into active molecules with longer half-lives than their parent compounds. Nitroglycerin is denitrated into glyceryl 1,2-dinitrate and glyceryl 1,3-dinitrate; these active metabolites have a half-life of approximately 40 minutes. Isosorbide dinitrate is denitrated into isosorbide 2-mononitrate and isosorbide 5-mononitrate; these active metabolites have half-lives of 2 and 4 hours, respectively.

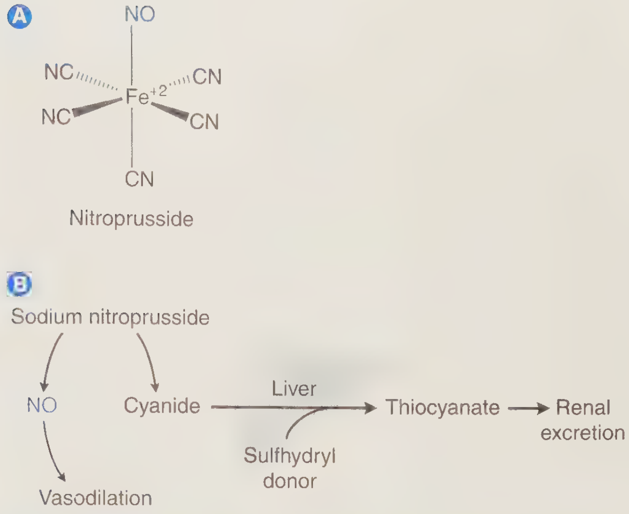


**Figure 20-10. Chemical Structure and Metabolism of Sodium Nitroprusside.** **A,** Sodium nitroprusside is a complex of iron, cyanide (CN), and a nitroso (NO) group. **B,** Sodium nitroprusside is metabolized to release NO and cyanide. NO effects vasodilation; cyanide is metabolized in the liver to thiocyanate, which undergoes renal excretion. Cyanide toxicity can result upon prolonged administration of the drug or in the presence of renal insufficiency.

a longer half-life (about 1 hour). The partially denitrated metabolites of isosorbide dinitrate––isosorbide 2-mononitrate and isosorbide 5-mononitrate—have even longer half-lives (up to 2 and 4 hours, respectively) (Fig. 20-9). **Isosorbide 5-mononitrate** has itself become a popular therapeutic agent, not only because it has prolonged therapeutic effects, but also because it is well-absorbed from the gastrointestinal tract and is not susceptible to extensive first-pass metabolism in the liver. The bioavailability of orally administered isosorbide 5-mononitrate is nearly 100%, allowing isosorbide 5-mononitrate to be significantly more effective than equivalent amounts of isosorbide dinitrate. After denitration, organic nitrates are typically glucuronidated in the liver and excreted renally.

## Pharmacologic Tolerance

The desirable effects of nitrates can, unfortunately, be offset by compensatory sympathetic nervous system responses (e.g., a reflex increase in sympathetic vascular tone) and compensatory renal responses (e.g., increased salt and water retention). In addition to these **physiologic tolerance** mechanisms, **pharmacologic tolerance** to organic nitrates is an important and clinically relevant phenomenon that significantly limits the efficacy of this class of vasodilators. Pharmacologic tolerance was first documented in munitions workers exposed to volatile organic nitrates in the workplace. These workers suffered severe headaches at the start of the workweek, but as the week progressed, the headaches tended to disappear and remain absent for the rest of the workweek. Upon returning to work after a weekend without nitrate exposure, however, the headaches recurred. These "Monday morning headaches" were initially ascribed to weekend intemperance, but it later became clear that the vasodilatory effects of NTG were responsible. Development of tolerance to NTG as the workweek progressed allowed relief from the headaches, and loss of tolerance to NTG over the weekend allowed the headaches to recur upon returning to work.

Although tolerance to such side effects as headaches can be desirable, *tolerance to the antianginal effects of nitrates diminishes their clinical efficacy.* Tolerance to NTG does not seem to depend on the route of administration. Importantly, it is possible to minimize the development of tolerance by modulating the dosing schedule to include daily "nitrate-free intervals." For transdermal NTG, simple removal of the NTG patch each night can minimize the development of tolerance. In cases of severe angina that require uninterrupted nitrate therapy to manage symptoms adequately, however, patients may have rebound angina during periods that are completely nitrate-free. The pharmacokinetic properties of oral isosorbide 5-mononitrate make this preparation an attractive solution to the dilemma of balancing nitrate tolerance and anginal rebound: Its high bioavailability and long half-life produce periods of high-therapeutic plasma concentrations followed by periods of low-therapeutic (rather than zero) nitrate levels. The examples of transdermal NTG and oral isosorbide 5-mononitrate illustrate how the pharmacokinetic properties of two mechanistically similar drugs can have significant impact on their therapeutic utility.

The cellular and molecular mechanisms that underlie the development of pharmacologic tolerance to organic nitrates remain unclear. There are currently two major hypotheses. First, the so-called classic (sulfhydryl) theory suggests that tolerance results mainly from intracellular depletion of sulfhydryl-containing groups, such as glutathione and/or other forms of cysteine, that are involved in the formation of NO from organic nitrates (Fig. 20-8). According to the sulfhydryl hypothesis, tolerance could be attenuated or reversed by administering **reduced thiol-containing compounds** such as ***N*-acetylcysteine.** Second, the free radical (superoxide) hypothesis posits that cellular tolerance results from the formation of **peroxynitrite,** a highly reactive metabolite of NO that seems to inhibit guanylyl cyclase. According to the superoxide theory, tolerance could be attenuated or reversed by agents that inhibit free radical formation. Because the specific mechanisms of nitrate tolerance remain uncertain, the most effective means of preventing tolerance is the use of a dosing strategy that includes an interval of low plasma nitrate levels every day.

### Effects of Nitrates in Addition to Vasodilation

The generation of NO from organic nitrates can cause relaxation of other types of smooth muscle—such as esophageal, bronchial, biliary, intestinal, and genitourinary—in addition to vascular smooth muscle. Indeed, *the ability of NTG to relieve the angina-like chest pain of esophageal spasm can occasionally result in a misdiagnosis of angina.* These actions of nitrates on nonvascular smooth muscle are usually of limited clinical significance, however.

NO generated from organic nitrates functions as an antiplatelet agent as well as a vascular smooth muscle relaxant. NO-mediated increases in platelet cGMP inhibit platelet aggregation; together with the vasodilatory effect of the nitrates, this antiplatelet effect may decrease the likelihood of coronary artery thrombosis. Nitrate-induced inhibition of platelet aggregation may be especially important in the treatment of **rest angina** (i.e., chest pain that occurs spontaneously at rest), because rest angina frequently results from occlusive platelet aggregation near atherosclerotic coronary lesions. Rest angina is also known as **unstable angina** because the thrombotic occlusions that cause rest angina often evolve into complete coronary occlusion and result in myocardial infarction.

### Contraindications

As discussed above, nitrates are contraindicated in patients with hypotension. Nitrates are also contraindicated in patients with elevated intracranial pressure, because NO-mediated vasodilation of cerebral arteries could further elevate intracranial pressure. Nitrates are not advised for the anginal pain associated with hypertrophic obstructive cardiomyopathy because the obstruction can be worsened by nitrate-mediated preload reduction. Nitrates should also be used with caution in patients with diastolic heart failure, who depend on an elevated ventricular preload for optimal cardiac output. A more recently discovered contraindication to nitrate use is in patients taking sildenafil (Viagra). GF's case provides an example of the deleterious effects of concurrently administered organic nitrates and sildenafil (see below).

## PHOSPHODIESTERASE INHIBITORS

Phosphodiesterase inhibitors prevent the hydrolysis of cyclic nucleotides (cAMP, cGMP) to their monophosphate forms (5′-AMP, 5′-GMP). Certain phosphodiesterase inhibitors—such as **amrinone** and **milrinone**—are selective for the isoforms of phosphodiesterase found mainly in cardiac and vascular smooth muscle and are discussed further in Chapter 18, Pharmacology of Cardiac Contractility.

**Sildenafil** is a phosphodiesterase inhibitor that is highly selective for **cGMP phosphodiesterase type V (PDE5).** PDE5 is found mainly in the smooth muscle of the corpus cavernosum, but is also expressed in the retina and in vascular smooth muscle cells. Sildenafil is commonly prescribed for **erectile dysfunction (ED),** a condition that is not uncommon in men, such as GF, who have vascular disease. In normal physiology, NO released from penile nerve terminals activates guanylyl cyclase in smooth muscle of the corpus cavernosum, leading to an increase in intracellular cGMP concentration, smooth muscle relaxation, inflow of blood, and penile erection. Because it inhibits the cGMP phosphodiesterase in the corpus cavernosum smooth muscle, sildenafil can potentiate the effects of endogenous NO-cGMP signaling and, thereby, potentiate penile erection.

Although PDE5 is expressed predominantly in erectile smooth muscle tissue, it is also expressed in small amounts in the systemic vasculature. Thus, although the majority of sildenafil's actions are highly localized, the drug can also attenuate cGMP hydrolysis in the systemic vasculature by inhibiting the small amounts of PDE5 present there. In this manner, the enhancement of endogenous NO-cGMP signaling by sildenafil can cause dilation of vascular smooth muscle. Several of sildenafil's adverse effects (e.g., headache and flushing) result from its vasodilatory properties. Sildenafil-related myocardial infarction and sudden cardiac death may also be related to its vasodilatory effects. These side effects are relatively rare because of the small amounts of PDE5 in vascular smooth muscle. However, in the presence of excess NO (e.g., when organic nitrates are used concomitantly with sildenafil), sildenafil's inhibition of cGMP degradation amplifies the vasodilatory effect. Excessive vasodilation can lead to severe refractory hypotension, as GF experienced after taking nitroglycerin and sildenafil at the same time.

Given the serious consequences of combining organic nitrates and sildenafil, one might wonder whether non-nitrate vasodilator agents are also contraindicated in patients, such as GF, who use sildenafil. In one preliminary study, the degree of blood pressure reduction in a cohort receiving both sildenafil and amlodipine, a vasodilatory $Ca^{2+}$ channel blocker, did not differ significantly from that in a cohort receiving sildenafil and a placebo. Nonetheless, patients taking vasodilatory antihypertensive medications together with sildenafil should be regarded as at risk for potentially dangerous hypotension. Theoretically, the risk could be even greater for patients taking sildenafil in conjunction with both an antihypertensive vasodilator and a drug that inhibits sildenafil's degradation by hepatic CYP3A4 (see Chapter 4, Drug Metabolism).

## CA$^{2+}$ CHANNEL BLOCKERS

In contrast to organic nitrates, which have pharmacologic effects limited mostly to the vasculature, $Ca^{2+}$ channel blockers act on both vascular smooth muscle and the myocardium. Another important difference between organic nitrates and $Ca^{2+}$ channel blockers is that, while organic nitrates have mainly venodilatory activity, $Ca^{2+}$ channel blockers are predominantly arteriolar dilators. $Ca^{2+}$ channel blockers are commonly used in the treatment of hypertension, certain cardiac arrhythmias, and some forms of angina.

### Mechanism of Action

Several different subtypes of voltage-gated $Ca^{2+}$ channels have been identified (termed L, T, N, and P channels). These subtypes differ in their electrochemical and biophysical properties and in their tissue distribution patterns. $Ca^{2+}$ influx through the L-type channel is an important determinant of vascular tone and cardiac contractility. The $Ca^{2+}$ channel blockers in current use all act by inhibiting $Ca^{2+}$ entry through the L-type channel, although different members of this drug class have markedly different pharmacodynamic and pharmacokinetic properties.

In smooth muscle cells, decreased $Ca^{2+}$ entry through L-type channels keeps $[Ca^{2+}]_{intracellular}$ low, thereby reducing $Ca^{2+}$/CaM-mediated activation of myosin light chain kinase, actin–myosin interaction, and smooth muscle contractility (Fig. 20-3). Although $Ca^{2+}$ channel blockers can relax many different types of smooth muscle (e.g., bronchiolar and gastrointestinal), they seem to have the greatest effect on vascular smooth muscle. In addition, the arterial smooth muscle is more responsive than the venous smooth muscle. Vasodilation of resistance arterioles reduces systemic vascular resistance and lowers arterial blood pressure, thereby decreasing ventricular systolic wall stress and myocardial $O_2$ demand. Dilation of coronary arteries may also increase myocardial $O_2$ supply, further ameliorating the $O_2$ supply: demand mismatch in patients with angina. In cardiac myocytes, reduced $Ca^{2+}$ influx through L-type channels leads to decreases in myocardial contractility, sinoatrial (SA) node pacemaker rate, and atrioventricular (AV) node conduction velocity (see Chapter 17, Pharmacology of Cardiac Rhythm, for a discussion of the effects of $Ca^{2+}$ channel blockers on cardiac muscle).

It is important to note that skeletal muscle is not significantly affected by $Ca^{2+}$ channel blockers, because skeletal muscle depends mainly on intracellular pools of $Ca^{2+}$ (i.e., from the sarcoplasmic reticulum) to support excitation–contraction coupling and does not require as much transmembrane $Ca^{2+}$ influx through the L-type channel. One should also distinguish between smooth muscle relaxants that block $Ca^{2+}$ entry through the L-type $Ca^{2+}$ channel in vascular smooth muscle cells, and skeletal muscle relaxants that block neurotransmission mediated by the nicotinic acetylcholine receptor at the neuromuscular junction (see Chapter 7, Cholinergic Pharmacology).

### Chemical Classes

Three chemical classes of $Ca^{2+}$ channel blockers—the **dihydropyridines** (exemplified by nifedipine and amlodipine), the **benzothiazepines** (exemplified by diltiazem), and the **phenylalkylamines** (exemplified by verapamil)—are currently in clinical use. All three classes block the L-type $Ca^{2+}$ channel, but each class has distinctive pharmacologic effects. The differences are in part attributable to different drug binding sites on the $Ca^{2+}$ channel: Nifedipine binds to the N binding site, diltiazem binds to the D binding site, and verapamil binds to the V binding site. The D and V binding sites overlap, whereas, the N site is in a different region of the $Ca^{2+}$ channel. Diltiazem and verapamil affect each other's binding in complex ways, as might be expected for two drugs with overlapping binding sites. Nifedipine and diltiazem bind synergistically, whereas nifedipine and verapamil reciprocally inhibit each other's binding. An added layer of complexity arises from the different affinities of the $Ca^{2+}$ channel blockers for different conformational states of the channel. Perhaps because different channel conformations are preferred in different tissues, the drugs also have differential tissue selectivity.

**Nifedipine** and **amlodipine** are representative members of the dihydropyridine class of $Ca^{2+}$ channel blockers. Compared to the other $Ca^{2+}$ channel blockers, the dihydropyridines cause significantly more arterial vasodilation. In contrast, the dihydropyridines have relatively little effect on cardiac tissue. Compared to diltiazem and verapamil, the dihydropyridines cause less depression of myocardial contractility and have minimal effects on SA node automaticity and AV node conduction velocity (Fig. 20-11).

Amlodipine (a third-generation dihydropyridine) differs from nifedipine (a first-generation dihydropyridine) principally in its pharmacokinetic properties. Because amlodipine has a pKa of 8.7, it is predominantly in a positively charged form at physiologic pH. This positive charge allows amlodipine to bind to cell membranes (which are typically negatively charged) with high affinity, and contributes to the drug's late peak plasma concentration and slow hepatic metabolism (see below).

Compared to the dihydropyridines, **diltiazem** and **verapamil** have a lower ratio of vascular-to-cardiac selectivity (Table 20-2). In the heart, both diltiazem and verapamil act as negative inotropes; verapamil has a greater suppressive effect on cardiac contractility than diltiazem. Furthermore, because diltiazem and verapamil not only reduce the transmembrane influx of $Ca^{2+}$ but also slow the rate of $Ca^{2+}$ channel recovery, cardiac conduction (i.e., automaticity and conduction velocity) is significantly decreased by these drugs. (Dihydropyridines decrease the transmembrane influx of $Ca^{2+}$ but do not significantly alter the rate of $Ca^{2+}$ channel recovery, and, thus, have only minor effects on automaticity and conduction velocity.)

### Pharmacokinetics

$Ca^{2+}$ channel blockers are typically used as oral dosage forms, although intravenous formulations of diltiazem and verapamil also exist. Nifedipine and verapamil are excreted by the kidney; diltiazem is excreted by the liver. Several pharmacokinetic properties of these drugs are suboptimal.

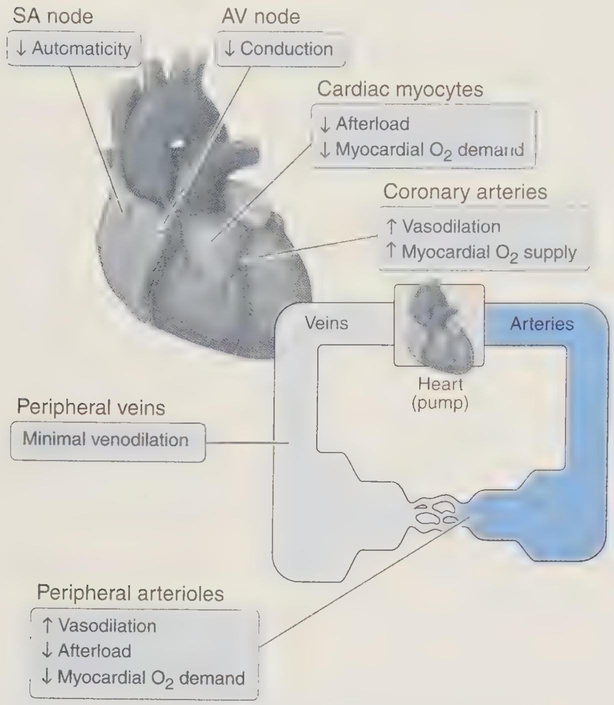


**Figure 20-11. Sites of Action of Ca$^{2+}$ Channel Blockers.** Ca$^{2+}$ channel blockers dilate coronary arteries and peripheral arterioles, but not veins. They also decrease cardiac contractility, automaticity at the SA node, and conduction at the AV node. Dilation of the coronary arteries increases myocardial $O_2$ supply. Dilation of systemic (peripheral) arterioles decreases afterload and, consequently, decreases myocardial $O_2$ demand. However, some Ca$^{2+}$ channel blockers (especially dihydropyridines) cause reflex tachycardia, which can paradoxically increase myocardial $O_2$ demand (not shown). Decreased cardiac contractility and decreased SA node automaticity also decrease myocardial $O_2$ demand. The inhibition of AV node conduction by some Ca$^{2+}$ blockers makes them useful as antiarrhythmic agents. Note that the effects diagrammed here are representative effects of the class of drugs; individual agents are more or less selective for each of these effects (see Table 20-2).

First, the bioavailability of oral formulations of nifedipine, diltiazem, and verapamil is lowered by significant first-pass metabolism in the gut and liver. Furthermore, oral nifedipine has a rapid onset of action (less than 20 min), and can cause a brisk, precipitous fall in blood pressure that can activate severe reflex tachycardia. In turn, reflex tachycardia can worsen myocardial ischemia by increasing myocardial $O_2$ demand and decreasing myocardial $O_2$ supply (the latter by decreasing diastolic filling time). In addition, the short half-life of oral nifedipine (about 4 h) requires that this drug be administered frequently.

As noted above, amlodipine and nifedipine share similar pharmacodynamic profiles, but differ significantly in their pharmacokinetic profiles. Amlodipine's high bioavailability permits it to be effective at lower dosages because a greater proportion of the administered drug reaches the systemic circulation unchanged. Amlodipine's late peak plasma concentration and slow onset of action may be the reason that, as compared to nifedipine, amlodipine causes significantly less reflex tachycardia. Slow hepatic degradation of amlodipine contributes to its long plasma half-life (about 40 h) and duration of action, which permit once-daily dosing.

### Toxicities and Contraindications

The toxicities of the Ca$^{2+}$ channel blockers are mainly mechanism-based and are, therefore, typically extensions of their actions. Flushing (a common side effect of nifedipine) and constipation (a common side effect of verapamil) are likely caused by excessive smooth muscle relaxation in the cutaneous vasculature and gastrointestinal tract, respectively. In excess, the negative chronotropic and negative inotropic effects of verapamil and diltiazem can lead to bradycardia, atrioventricular block, and congestive heart failure. Patients taking β-adrenergic blockers (which are also negative inotropes) are advised not to use Ca$^{2+}$ channel blockers concomitantly, because of the increased likelihood of excessive cardiac depression. Some studies have suggested that Ca$^{2+}$ channel blockers increase the risk of mortality in patients with congestive heart failure, and Ca$^{2+}$ channel blockers are, therefore, contraindicated in the management of congestive heart failure. Some reports also suggest that short-acting nifedipine is associated with an increased risk of myocardial ischemia and infarction, by virtue of this drug's tendency to disturb the myocardial $O_2$ supply:demand equilibrium (see above).

## K$^+$ CHANNEL OPENERS

K$^+$ channel openers cause direct arterial vasodilation by opening **metabotropic ATP-sensitive K$^+$ channels.** Because

**TABLE 20-2** Selectivity of Ca$^{2+}$ Channel Blockers

| | VASODILATION (PERIPHERAL ARTERIOLES AND CORONARY ARTERIES) | DEPRESSION OF CARDIAC CONTRACTILITY | DEPRESSION OF AUTOMATICITY (SA NODE) | DEPRESSION OF CONDUCTION (AV NODE) |
|---|---|---|---|---|
| Nifedipine | 5 | 1 | 1 | 0 |
| Diltiazem | 3 | 2 | 5 | 4 |
| Verapamil | 4 | 4 | 5 | 5 |

The effects of the three different classes of Ca$^{2+}$ channel blockers on vascular tone, cardiac contractility, heart rate, and AV nodal conduction are graded from 0 to 5. 0 = no effect; 5 = significant effect. Note that nifedipine is the most selective drug for peripheral vasodilation, while diltiazem and verapamil have more selective effects on the heart.

these agents act by a mechanism that is entirely different from that of other vasodilators, $K^+$ channel openers represent a potent family of drugs that can be used to treat hypertension refractory to other antihypertensive therapeutics.

What is the normal function of metabotropic $K^+$ channels? Recall that the Nernst equilibrium potential for $K^+$ is about –90 mV, while the resting membrane potential is less negative than this value. Therefore, opening $K^+$ channels *hyperpolarizes* the membrane. If a sufficient number of $K^+$ channels are open at the same time, then normal excitatory stimuli are not able to promote membrane depolarization. In the absence of depolarization, voltage-gated $Ca^{2+}$ channels do not open, and $Ca^{2+}$ influx and smooth muscle contraction are inhibited (Fig. 20-3).

$K^+$ channel opener drugs include **minoxidil, cromakalim, pinacidil,** and **nicorandil.** The $K^+$ channel openers act primarily on arterial smooth muscle cells and, therefore, decrease arterial blood pressure. Side effects of the $K^+$ channel openers include headache, caused by excessive dilation of cerebral arteries, and flushing, caused by excessive dilation of cutaneous arteries. When arterial vasodilators (e.g., $Ca^{2+}$ channel blockers or $K^+$ channel openers) are used as monotherapy, the decrease in arterial pressure often elicits reflex sympathetic discharge, leading to tachycardia and increased cardiac work. As noted above in the discussion of nifedipine, reflex sympathetic discharge can offset the balance between myocardial $O_2$ supply and demand, precipitating myocardial ischemia; this effect is of particular concern in patients with pre-existing coronary artery disease. However, the use of β-blockers in combination with arterial vasodilators can help to block the effects of reflex sympathetic activity and, thereby, preserve the therapeutic utility of the arterial vasodilators.

## OTHER DRUGS THAT MODULATE VASCULAR TONE

### Hydralazine

**Hydralazine** is an orally administered arteriolar vasodilator that is sometimes used in the treatment of hypertension and, in combination with isosorbide dinitrate, in the treatment of heart failure. The mechanism of action of hydralazine remains unclear, although different studies have suggested that membrane hyperpolarization, $K^+$ channel activation, and inhibition of $IP_3$-induced $Ca^{2+}$ release from the sarcoplasmic reticulum in vascular smooth muscle cells may all be involved. Hydralazine also seems to prevent the development of nitrate tolerance, perhaps by inhibiting vascular superoxide production. Indeed, the success of hydralazine–isosorbide dinitrate combination therapy for heart failure may be related to hydralazine's prevention of nitrate tolerance.

The use of hydralazine has been limited because it was initially thought that the frequent dosing required for sustained blood pressure control and the rapid development of tachyphylaxis to its antihypertensive effects made chronic use of this drug impractical. As the benefits of combination therapy for hypertension and heart failure are becoming better appreciated, it may be possible for hydralazine to be used more effectively, especially in patients for whom other vasodilators (e.g., ACE inhibitors) are contraindicated.

Hydralazine typically has low bioavailability because of extensive first-pass hepatic metabolism. The rate of its metabolism depends on whether the patient is a slow or fast acetylator, however. In slow acetylators (see Chapter 4), hydralazine has a slower rate of hepatic degradation and, thus, greater bioavailability and higher plasma concentrations. A rare side effect of hydralazine treatment is the development of a reversible lupus erythematosus-like syndrome; this side effect occurs chiefly in slow acetylators.

### $\alpha_1$-Adrenergic Antagonists

Epinephrine and norepinephrine stimulate **$\alpha_1$-adrenergic receptors** on vascular smooth muscle and, thereby, induce vasoconstriction. The $\alpha_1$-adrenergic receptor is a G protein-coupled receptor that associates with the heterotrimeric G protein $G_q$, which activates phospholipase C to generate inositol triphosphate and diacylglycerol. $\alpha_1$-Adrenergic antagonists, such as **prazosin,** block $\alpha_1$-adrenergic receptors in arterioles and venules, leading to vasodilation. The effect of these agents is greater in arterioles than in venules. The $\alpha_1$-adrenergic antagonists cause a significant reduction in arterial pressure and are, thus, useful in the treatment of hypertension. Initiation of therapy with $\alpha_1$-adrenergic antagonists can be associated with orthostatic hypotension. Like other arterial vasodilators, the $\alpha_1$-adrenergic antagonists can cause retention of salt and water. β-Adrenergic blockers and diuretics may be used together with $\alpha_1$-adrenergic antagonists to mitigate these compensatory responses. Some $\alpha_1$-adrenergic antagonists, such as **terazosin,** are used principally to inhibit the contraction of nonvascular smooth muscle (e.g., prostatic smooth muscle), but these agents also have some effect on the vasculature (see Chapter 8, Adrenergic Pharmacology).

### β-Adrenergic Antagonists

Activation of **$\beta_2$-adrenergic receptors** on vascular smooth muscle cells leads to vasodilation. The increased intracellular cAMP induced by $\beta_2$-receptor stimulation may cause smooth muscle relaxation by accelerating the inactivation of myosin light chain kinase and by increasing the extrusion of $Ca^{2+}$ from the cell. Despite the beneficial vasodilatory effects of $\beta_2$-agonist action in the systemic circulation, β-adrenergic *antagonists* are of major clinical importance in the treatment of hypertension, angina, cardiac arrhythmias, and other conditions. β-Adrenergic antagonists have negative inotropic and chronotropic effects on the heart; these actions serve to reduce cardiac output, which is an important variable in determining both myocardial $O_2$ demand and blood pressure (see Equation 20-2). The cardiac effects of β-adrenergic antagonists are discussed in greater detail in Chapter 18. These drugs also have important effects on the vasculature: antagonism of $\beta_2$-adrenergic receptors on vascular smooth muscle cells can lead to unopposed vasoconstriction mediated by $\alpha_1$-adrenergic receptors and, consequently, to an increase in systemic vascular resistance. Importantly, although some β-adrenergic antagonists may initially increase systemic vascular resistance, the net effect in most cases is a *decrease* in blood pressure. This hypotensive effect reflects the combined negative inotropic effect (leading to a decrease in cardiac output), inhibition of renin secretion, and CNS effects of the β-blockers. Indeed, β-adrenergic antagonists are highly effective in the treatment of hypertension.

## Renin–Angiotensin System Blockers

As discussed in Chapter 19, inhibition of the renin–angiotensin system results in significant vasorelaxation. The hypotensive effect of ACE inhibitors may be caused by decreased catabolism of bradykinin, a vasorelaxant released in response to inflammatory stimuli. Antagonists of the $AT_1$ receptor, which selectively inhibit angiotensin II-mediated vasoconstriction at the level of the target organ, have a more direct effect. ACE inhibitors and $AT_1$ receptor antagonists are considered "balanced" vasodilators because they affect both arterial and venous vasodilation. Both classes of drugs are effective in the treatment of hypertension and congestive heart failure, as discussed in Chapter 23.

## *Conclusion and Future Directions*

Vascular tone represents a balance between vascular smooth muscle contraction and relaxation. Vasoconstriction occurs when an increase in intracellular $Ca^{2+}$ activates $Ca^{2+}$/CaM-dependent myosin light chain kinase that, in turn, phosphorylates myosin light chains and allows the formation of actin–myosin cross-bridges. Vascular smooth muscle relaxes when the intracellular $Ca^{2+}$ concentration returns to basal levels and myosin light chains are dephosphorylated, terminating the formation of actin–myosin cross bridges. Vascular tone is influenced by the state of the vascular smooth muscle cells and the overlying endothelial cells, by sympathetic innervation, and by neurohormonal and local regulators. Classes of vasodilators include nitrates, $Ca^{2+}$ channel blockers, $K^+$ channel openers, $\alpha_1$-adrenergic receptor antagonists, ACE inhibitors, $AT_1$ receptor antagonists, and endothelin receptor antagonists (Fig. 20-6). Nitrates dilate primarily veins, not arteries. These agents act by releasing NO in vascular smooth muscle cells; in turn, NO activates guanylyl cyclase, which increases intracellular cGMP, which activates cGMP-dependent protein kinase, which activates myosin light chain phosphatase, which terminates the formation of actin–myosin cross-bridges. $Ca^{2+}$ channel blockers act mainly on arteries and resistance arterioles, and may also have direct effects on the heart. These drugs cause vasodilation by blocking L-type voltage-gated $Ca^{2+}$ channels in the plasma membrane of vascular smooth muscle cells and, thereby, inhibiting the $Ca^{2+}$ influx through these channels that is necessary for contraction. $K^+$ channel openers, as with $Ca^{2+}$ channel blockers, are predominantly arteriodilators, not venodilators. This class of drugs opens metabotropic ATP-sensitive $K^+$ channels, which action hyperpolarizes vascular smooth muscle cells and prevents the activation of voltage-gated $Ca^{2+}$ channels that is necessary for $Ca^{2+}$ influx and muscle contraction. $\alpha_1$-Adrenergic receptor antagonists, $AT_1$ receptor antagonists, and endothelin receptor antagonists prevent vasoconstriction by inhibiting the activation of their respective receptors by endogenous agonists. In summary, vascular tone is subject to exquisite control, as would be expected for a system that must perfuse all the tissues of the body. Diverse therapeutic agents modulate various components of this critical system, with important differences in molecular mechanisms.

Still more classes of vasodilators are under active clinical development. $ET_A$-selective, $ET_B$-selective, and nonselective endothelin receptor antagonists have recently been developed. Drug trials are currently underway to evaluate the utility of these agents in the treatment of such cardiovascular diseases as hypertension, atherosclerosis, and heart failure. Given the important role of natriuretic peptides such as BNP in regulating vascular tone and intravascular volume, modulators of natriuretic peptides and their receptors may also have therapeutic potential for such cardiovascular conditions as congestive heart failure, acute myocardial infarction, and hypertension. Natriuretic peptide receptor agonists are attractive therapeutic agents because: 1) they cause vasodilation without reflex activation of the sympathetic nervous system; and 2) they cause natriuresis without reflex activation of the renin–angiotensin system. One interesting pharmacologic approach to augmenting the actions of endogenous natriuretic peptides involves drug-induced inhibition of natriuretic peptide metabolism. Neutral endopeptidase inhibitors are currently under investigation as possible treatments for congestive heart failure and systemic hypertension.

## *Suggested Readings*

Berne RM, Levy MN. The peripheral circulation and its control. In: Cardiovascular Physiology. 7th ed. New York: Mosby, 1997: 171–204. (*Excellent reference on the normal physiology of vascular tone*)

deLemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003;362:316–322. (*Concise review of the physiology and pharmacology of natriuretic peptides*)

Michel T, Weinfeld MS. Stable coronary artery disease: Angina and atherosclerosis. In: Melmon and Morrelli's Clinical Pharmacology: Basic Principles in Therapeutics. 4th ed. Melmon KL et al., eds. New York: McGraw Hill, 2000:114–131. (*Contains many helpful diagrams and tables and discusses important issues in the use of vasodilating agents to treat coronary artery disease*)

Opie L, Gersh BJ. Drugs for the Heart. 5th ed. Philadelphia: WB Saunders, 2001. (*A "pocket-sized" clinician's guide with diagrams*)

Parker JD, Parker JO. Nitrate therapy for stable angina pectoris. N Engl J Med 1998;338:520–531. (*Excellent reference on the pharmacokinetics and tolerance mechanisms of the organic nitrates*)

## Drug Summary Table Pharmacology of Vascular Tone

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ORGANIC NITRATES AND SODIUM NITROPRUSSIDE**<br>*Mechanism—Donate NO, which activates guanylyl cyclase and increases dephosphorylation of myosin light chain in vascular smooth muscle, causing vessel dilation* | | | | |
| **Nitroglycerin** | Short-acting (sublingual, spray): Acute effort angina<br>Long-acting (oral, buccal, transdermal patch): Angina prophylaxis<br>Intravenous: Unstable angina, acute heart failure | All organic nitrates can cause:<br>Flushing<br>Headache<br>Refractory hypotension<br>Syncope<br>Tachycardia<br>Angina can be provoked if hypotension leads to decreased coronary perfusion and provokes reflex tachycardia | For all organic nitrates:<br>Use cautiously in patients with hypotension, elevated intracranial pressure, hypertrophic obstructive cardiomyopathy, diastolic heart failure<br>Concurrent PDE5 inhibitor use may lead to excessive hypotension and possible myocardial infarction | Venous dilation > arteriolar dilation<br>Continuous therapy leads to tolerance. Tolerance can be avoided by providing nitrate-free intervals |
| **Isosorbide dinitrate** | Short-acting (sublingual): Acute effort angina<br>Long-acting (oral): Angina prophylaxis, heart failure | | | |
| **Isosorbide 5-mononitrate** | Angina prophylaxis | | | Venous dilation > arteriolar dilation<br>Popular because it is well absorbed from the GI tract, relatively unaffected by first-pass metabolism, and has prolonged therapeutic effects<br>Less tolerance and rebound of anginal symptoms than other organic nitrates |
| **Sodium nitroprusside** | Intravenous only: Hypertensive emergencies, severe cardiac failure | Cyanide toxicity | Contraindicated in patients with preexisting hypotension, obstructive valvular disease, or hepatic or renal failure (impaired clearance of toxic metabolites) | Venous dilation = arteriolar dilation<br>Potency, rapid onset of action, and short duration of action require continuous blood pressure monitoring and careful titration |
| **PHOSPHODIESTERASE TYPE V INHIBITORS**<br>*Mechanism—Inhibit PDE5, an enzyme that converts cGMP to GMP, leading to cGMP accumulation in target tissues* | | | | |
| **Sildenafil**<br>**Vardenafil**<br>**Tadalefil** | All PDE5 inhibitors can be used in:<br>Erectile dysfunction<br>Pulmonary hypertension? | All PDE5 inhibitors can cause:<br>Flushing<br>Headache<br>Refractory hypotension<br>Myocardial infarction<br>Sudden cardiac death<br>Blue vision (due to alteration of retinal cGMP metabolism)<br>Priapism | For all PDE5 inhibitors, concurrent use of organic nitrates may lead to refractory hypotension and myocardial infarction | Other phosphodiesterase inhibitors are discussed in Chapter 18<br>Tadalefil has 36-hour duration of action |

*(Continued)*

**Drug Summary Table** **Pharmacology of Vascular Tone** *(Continued)*

| Drug | Clinical Use | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **Ca²⁺ CHANNEL BLOCKERS**<br>*Mechanism—Block voltage-gated Ca²⁺ channels and prevent the influx of Ca²⁺ that promotes actin-myosin interactions (in vascular smooth muscle cells) and supports the cardiac action potential (in AV nodal cells)* | | | | |
| **Nifedipine**<br>**Amlodipine** | Ca²⁺ channel blockers can be used in:<br>Effort angina<br>Unstable angina<br>Coronary spasm<br>Hypertension<br>Raynaud's syndrome (nifedipine and amlodipine)<br>Supraventricular tachycardia (diltiazem and verapamil) | Nifedipine can cause:<br>Flushing<br>Headache<br>Rapid vasodilation → reflex tachycardia (→ myocardial infarction)<br>Amlodipine can cause:<br>Flushing<br>Dizziness<br>Peripheral edema | Nifedipine and amlodipine are contraindicated in patients with preexisting hypotension | Arteriolar dilation > venous dilation<br>High vascular-to-cardiac selectivity<br>Pharmacokinetic profile of amlodipine is distinguished by high bioavailability, late peak plasma concentration, and slow hepatic metabolism |
| **Diltiazem**<br>**Verapamil** | | Diltiazem and verapamil can cause:<br>Bradycardia<br>AV block<br>Cardiac arrest<br>Congestive heart failure<br>Constipation (verapamil) | Diltiazem and verapamil are contraindicated in patients with sick sinus syndrome, high-grade AV block, excess β-blockade, systolic heart failure | Low vascular-to-cardiac selectivity |
| **K⁺ CHANNEL OPENERS**<br>*Mechanism—Open metabotropic K⁺ channels and prevent depolarization of plasma membrane and, thus, activation of voltage-gated Ca²⁺ channels* | | | | |
| **Minoxidil**<br>**Cromakalim**<br>**Pinacidil**<br>**Nicorandil** | Hypertension refractory to other antihypertensive agents | Flushing<br>Headache<br>Rapid vasodilation → reflex tachycardia<br>Hirsutism (minoxidil only)<br>Peripheral edema | | Arteriolar dilation > venous dilation<br>Typically used in combination with a β-blocker and a diuretic |

|  |  |  |  |  |
|---|---|---|---|---|
| **HYDRALAZINE**<br>*Mechanism—Uncertain* |  |  |  |  |
| **Hydralazine** | Used in combination therapy for hypertension and congestive heart failure | Reversible lupus erythematosus-like syndrome (mainly in slow acetylators) |  | Arteriolar dilation > venous dilation<br>Typically used in combination with a β-blocker and a diuretic for hypertension<br>Used in combination with isosorbide dinitrate for congestive heart failure<br>QID dosing impairs patient compliance |
| **$\alpha_1$-ADRENERGIC ANTAGONISTS**<br>*Mechanism—Block activation of $\alpha_1$-adrenergic receptors* |  |  |  |  |
| **Prazosin**<br>**Doxazosin**<br>**Terazosin** | Hypertension<br>Benign prostatic hypertrophy | Postural hypotension with initial therapy |  | Arteriolar dilation > venous dilation<br>Typically used in combination with a β-blocker and a diuretic for hypertension |
| **β-ADRENERGIC ANTAGONISTS (ALSO, SEE CHAPTER 18)**<br>*Mechanism—Block activation of β-adrenergic receptors* |  |  |  |  |
| **Propranolol** (nonselective)<br>**Atenolol, metoprolol** ($\beta_1$-selective) | Hypertension<br>Angina<br>Acute myocardial infarction<br>Cardiac arrhythmias<br>CHF<br>Essential tremor<br>Thyrotoxicosis<br>Glaucoma | Smooth muscle spasm (e.g., bronchospasm)<br>Bradycardia<br>AV block<br>Insomnia, depression<br>Hyperlipidemia | Contraindicated in patients with sick sinus syndrome, high-grade AV block, decompensated systolic heart failure, severe asthma, severe depression, Raynaud's phenomenon, diabetes mellitus | In the vasculature, blockade of $\beta_2$-adrenergic receptors leads to unopposed vasoconstriction mediated by $\alpha_1$-adrenergic receptors<br>β -selective antagonists cause less smooth muscle spasm than nonselective antagonists do |
| **ACE INHIBITORS**<br>*Mechanism—Prevent angiotensin converting enzyme from cleaving ATI to ATII and from degrading bradykinin* |  |  |  |  |
| **Captopril**<br>**Enalapril**<br>**Lisinopril** | Hypertension<br>Acute myocardial infarction<br>Postinfarct therapy<br>Congestive heart failure<br>Diabetic nephropathy | Cough (common)<br>Angioedema (rare, but can be fatal)<br>Hypotension<br>Hyperkalemia<br>Renal failure | Contraindicated in pregnancy, bilateral renal artery stenosis, severe renal failure, hypotension, hyperkalemia |  |
| **$AT_1$ RECEPTOR ANTAGONISTS**<br>*Mechanism—Block activation of $AT_1$ receptors* |  |  |  |  |
| **Losartan**<br>**Valsartan** | Hypertension<br>Congestive heart failure |  | Contraindicated in pregnancy and bilateral renal artery stenosis |  |

# 21

# Pharmacology of Hemostasis and Thrombosis

April Wang Armstrong and David E. Golan

- **Introduction**
- **Case**
- **Physiology of Hemostasis**
  - Vasoconstriction
  - Primary Hemostasis
    - *Platelet Adhesion*
    - *Platelet Granule Release Reaction*
    - *Platelet Aggregation and Consolidation*
  - Secondary Hemostasis: The Coagulation Cascade
  - Regulation of Hemostasis
- **Pathogenesis of Thrombosis**
  - Endothelial Injury
  - Abnormal Blood Flow
  - Hypercoagulability
- **Pharmacologic Classes and Agents**
  - Antiplatelet Agents
    - *Cyclooxygenase Inhibitors*
    - *Phosphodiesterase Inhibitors*
    - *ADP Receptor Pathway Inhibitors*
    - *GPIIb–IIIa Antagonists*
  - Anticoagulants
    - *Immediate-Acting, Parenteral Anticoagulants: Unfractionated and Low Molecular Weight Heparins*
    - *Delayed-Acting, Oral Anticoagulants: Warfarin*
    - *Direct Thrombin Inhibition: Hirudin*
    - *Recombinant Activated Protein C (r-APC)*
  - Thrombolytic Agents
    - *Streptokinase*
    - *Urokinase*
    - *Recombinant Tissue Plasminogen Activator (t-PA)*
    - *Anisoylated Plasminogen Streptokinase Activator Complex (APSAC)*
- **Conclusion and Future Directions**
- **Suggested Readings**

## INTRODUCTION

Blood carries oxygen and nutrients to tissues and takes metabolic waste products away from tissues. Humans have developed a well-regulated system of **hemostasis** to keep the blood fluid and clot-free in normal vessels and to form a localized plug rapidly in injured vessels. **Thrombosis** describes a pathologic state in which normal hemostatic processes are activated inappropriately. For example, a blood clot (thrombus) may form as a result of a relatively minor vessel injury and occlude a section of the vascular tree. This chapter presents the normal physiology of hemostasis, the pathophysiology of thrombosis, and the pharmacology of drugs that can be used to prevent or reverse a thrombotic state. Drugs that are introduced in this chapter treat a variety of cardiovascular diseases, such as deep venous thrombosis and myocardial infarction.

## Case

Mr. Soprano, a 55-year-old man with a history of hypertension and cigarette smoking, is awakened in the middle of the night with substernal chest pressure, sweating, and shortness of breath. He calls 911 and is taken to the emergency room. An EKG shows deep T-wave inversions in leads V2–V5. A cardiac enzyme panel shows a creatine kinase level of 400 U/L (normal, < 200 U/L) with 10% MB fraction (the heart-specific isoform), suggesting myocardial infarction. He is treated with IV nitroglycerin, aspirin, unfractionated heparin, and eptifibatide, but his chest pain persists. He is taken to the cardiac catheterization laboratory, where he is found to have a 90% mid-LAD (left anterior descending artery) thrombus with sluggish distal flow. He undergoes successful angioplasty and stent placement. The heparin is stopped, the eptifibatide is continued for 18 more hours, and he is transferred to the telemetry ward. Six hours later, he is noted to have an expanding hematoma (an area of localized hemorrhage) in his right thigh below the arterial access site. The eptifibatide is stopped and pressure is applied to the access site, and the hematoma ceases to expand. He is discharged 2 days later with prescriptions for clopidogrel and aspirin, which are administered to prevent subacute thrombosis of the stent.

## QUESTIONS

1. How did a blood clot arise in Mr. Soprano's LAD?
2. If low molecular weight heparin had been used instead of unfractionated heparin, how would the monitoring of the patient's coagulation status during the procedure have been affected?
3. What accounts for the efficacy of eptifibatide (a platelet GPIIb–IIIa antagonist) in inhibiting platelet aggregation?
4. When the expanding hematoma was observed, could any measure other than stopping the eptifibatide have been used to reverse the effect of this agent?
5. How do aspirin, heparin, and eptifibatide act in the attempt to treat Mr. Soprano's blood clot and to prevent recurrent thrombus formation?

## PHYSIOLOGY OF HEMOSTASIS

An injured blood vessel must induce the formation of a blood clot to prevent blood loss and to allow healing. Clot formation must also remain localized to prevent widespread clotting within intact vessels. The formation of a localized clot at the site of vessel injury is accomplished in four steps (Fig. 21-1). First, **localized vasoconstriction** occurs as a response to a reflex neurogenic mechanism and to the secretion of endothelium-derived vasoconstrictors such as endothelin. Immediately following vasoconstriction, **primary hemostasis** occurs. During this stage, platelets are activated and adhere to the exposed subendothelial matrix. **Platelet activation** involves both a change in shape of the platelet and the release of secretory granule contents from the platelet. The secreted granule substances recruit other platelets, causing more platelets to adhere to the subendothelial matrix and to aggregate with one another at the site of vascular injury. Primary hemostasis ultimately results in the formation of a **primary hemostatic plug.**

The goal of the final two stages of hemostasis is to form a stable, permanent plug. During **secondary hemostasis,** also known as the **coagulation cascade,** the activated endothelium and other nearby cells (see below) express a membrane-bound procoagulant factor called tissue factor, which complexes with coagulation factor VII to initiate the coagulation cascade. The end result of this cascade is the activation of thrombin, a critical molecule. Thrombin serves two pivotal functions in hemostasis: (1) it converts soluble fibrinogen to an insoluble fibrin polymer that forms the matrix of the clot; and (2) it induces more platelet recruitment and activation. During the final stage, platelet aggregation and fibrin polymerization lead to the formation of a stable, **permanent plug.** In addition, **antithrombotic mechanisms** restrict the permanent plug to the site of vessel injury, ensuring that the permanent plug does not inappropriately extend to occlude the vascular tree.

## VASOCONSTRICTION

Immediately after vascular injury, transient arteriolar vasoconstriction occurs. This vasoconstriction is mediated by a poorly understood reflex neurogenic mechanism. Local endothelial secretion of endothelin, a potent vasoconstrictor, potentiates the reflex vasoconstriction. Because the vasoconstriction is transient, bleeding would continue if primary hemostasis were not activated.

## PRIMARY HEMOSTASIS

The goal of primary hemostasis is to form a platelet plug that rapidly (but temporarily) stabilizes vascular injury. Platelets play a pivotal role in primary hemostasis. **Platelets** are cell fragments that arise by budding from megakaryocytes in the bone marrow; these small, membrane-bound discs contain cytoplasm but lack nuclei. Glycoprotein receptors present in the platelet plasma membrane are the primary mediators by which platelets are activated. Primary hemostasis involves the transformation of platelets into a hemostatic plug through three reactions: (1) adhesion; (2) the granule release reaction; and (3) aggregation and consolidation.

### Platelet Adhesion

In the first reaction, platelets adhere to subendothelial collagen that is exposed after vascular injury (Fig. 21-2). This adhesion is mediated by **von Willebrand factor (vWF),** a large multimeric protein that is secreted by both activated platelets and the injured endothelium. vWF binds both to surface receptors (especially glycoprotein Ib (GPIb)) on the platelet membrane and to the exposed collagen; this "bridging" action mediates adhesion of platelets to the collagen. The GPIb:vWF:collagen interaction is critical for initiation of primary hemostasis, because it is the only known molecular mechanism by which platelets can adhere to the injured vessel wall.

### Platelet Granule Release Reaction

Adherent platelets undergo a process of activation (Fig. 21-3) during which the cells' granule contents are released. The release reaction is initiated by agonist binding to cell surface receptors, which activates intracellular protein phosphorylation cascades and ultimately causes release of granule contents. Specifically, stimulation by ADP, epinephrine, and collagen leads to activation of platelet membrane phospholipase $A_2$ ($PLA_2$). $PLA_2$ cleaves membrane phospholipids and liberates **arachidonic acid,** which is converted into a **cyclic endoperoxide** by platelet cyclooxygenase. Thromboxane synthase subsequently converts the cyclic endoperoxide into **thromboxane $A_2$ ($TxA_2$).** $TxA_2$, via a G protein-coupled receptor, causes vasoconstriction at the site of vascular injury by inducing a decrease in cAMP levels within vascular smooth muscle cells. $TxA_2$ also stimulates the granule **release reaction** within platelets, thereby propagating the cascade of platelet activation and vasoconstriction.

During the release reaction, large amounts of ADP, $Ca^{2+}$, ATP, serotonin, vWF, and platelet factor 4 are *actively*

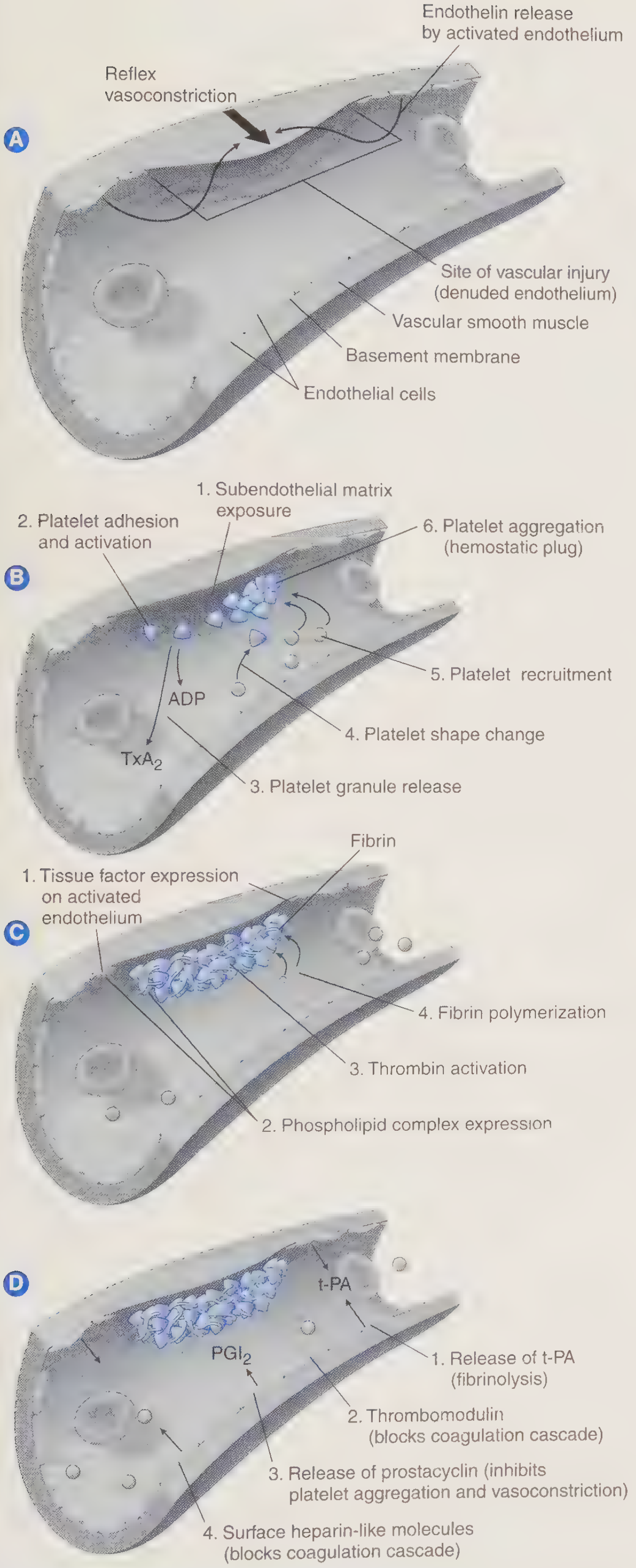

A
Reflex vasoconstriction
Endothelin release by activated endothelium
Site of vascular injury (denuded endothelium)
Vascular smooth muscle
Basement membrane
Endothelial cells
B
2. Platelet adhesion and activation
1. Subendothelial matrix exposure
6. Platelet aggregation (hemostatic plug)
ADP
TxA2
5. Platelet recruitment
4. Platelet shape change
3. Platelet granule release
C
1. Tissue factor expression on activated endothelium
Fibrin
4. Fibrin polymerization
3. Thrombin activation
2. Phospholipid complex expression
D
t-PA
PGI2
1. Release of t-PA (fibrinolysis)
2. Thrombomodulin (blocks coagulation cascade)
3. Release of prostacyclin (inhibits platelet aggregation and vasoconstriction)
4. Surface heparin-like molecules (blocks coagulation cascade)

**Figure 21-1. Sequence of Events in Hemostasis.** The hemostatic process can be divided into four steps: vasoconstriction, primary hemostasis, secondary hemostasis, and resolution. **A,** Vascular injury causes endothelial denudation. Endothelin, released by activated endothelium, and neurohumoral factor(s) induce transient vasoconstriction. **B,** Injury-induced exposure of the subendothelial matrix (1) provides a substrate for platelet adhesion and activation (2). In the granule release reaction, activated platelets secrete thromboxane $A_2$ ($TxA_2$) and ADP (3). $TxA_2$ and ADP released by activated platelets cause nearby platelets to become activated; these newly activated platelets undergo shape change (4) and are recruited to the site of injury (5). The aggregation of activated platelets at the site of injury forms a primary hemostatic plug (6). **C,** Tissue factor expressed on activated endothelial cells (1), together with acidic phospholipids expressed on activated platelets and activated endothelial cells (2), initiate the steps of the coagulation cascade, culminating in the activation of thrombin (3). Thrombin proteolytically activates fibrinogen to form fibrin, which polymerizes around the site of injury, resulting in the formation of a definitive (secondary) hemostatic plug (4). **D,** Natural anticoagulant and thrombolytic factors limit the hemostatic process to the site of vascular injury. These factors include tissue plasminogen activator (t-PA), which activates the fibrinolytic system (1); thrombomodulin, which activates inhibitors of the coagulation cascade (2); prostacyclin, which inhibits both platelet activation and vasoconstriction (3); and surface heparin-like molecules, which catalyze the inactivation of coagulation factors (4).

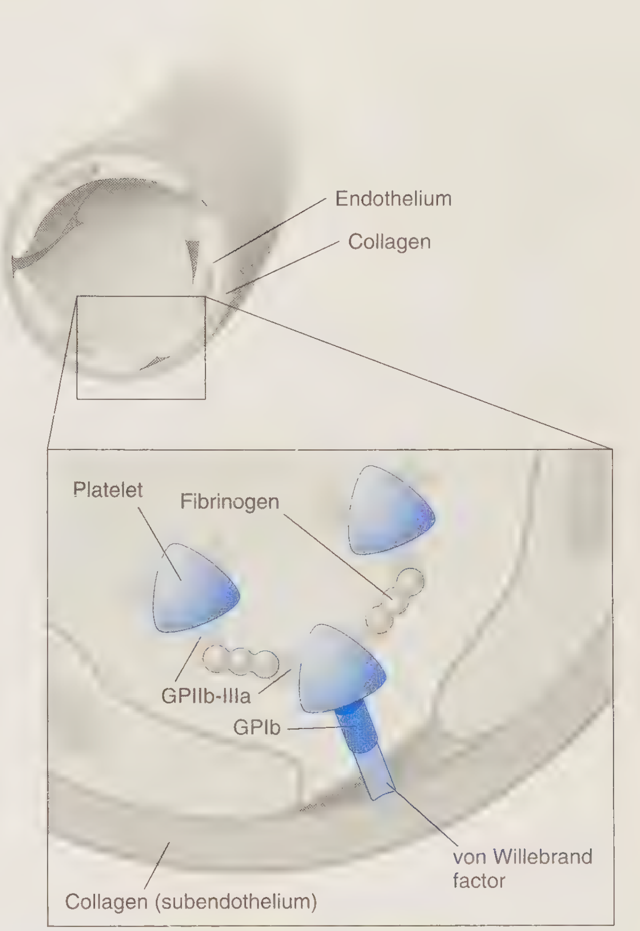

Endothelium
Collagen
Platelet
Fibrinogen
GPIIb-IIIa
GPIb
von Willebrand factor
Collagen (subendothelium)

**Figure 21-2. Platelet Adhesion and Aggregation.** von Willebrand factor mediates platelet adhesion to the subendothelium by binding both to the platelet membrane glycoprotein GPIb and to exposed subendothelial collagen. During platelet aggregation, fibrinogen crosslinks platelets to one another by binding to GPIIb–IIIa receptors on platelet membranes.

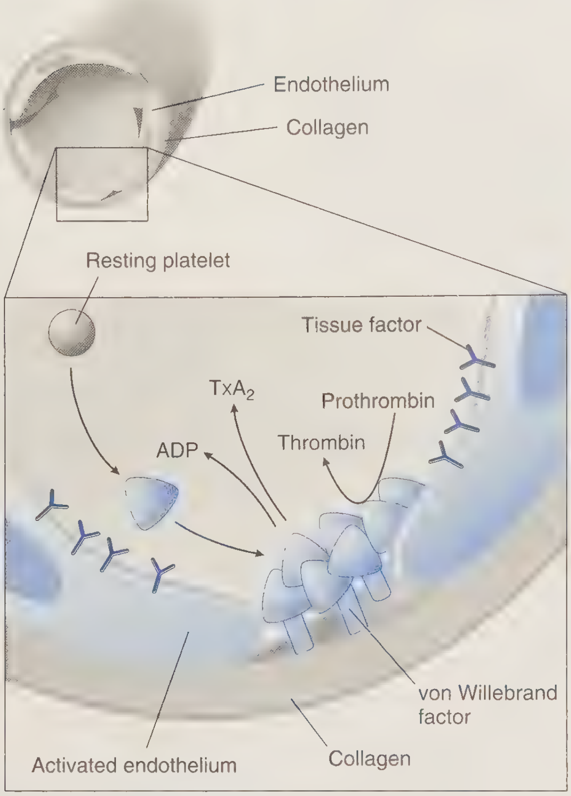


**Figure 21-3. Platelet Activation.** Platelet activation is initiated at the site of vascular injury when circulating platelets adhere to exposed subendothelial collagen and are activated by locally generated mediators. Activated platelets undergo shape change and granule release, and platelet aggregates are formed as additional platelets are recruited and activated. Platelet recruitment is mediated by the release of soluble platelet factors, including ADP and thromboxane $A_2$ ($TxA_2$). Tissue factor, expressed on activated endothelium, is a critical initiating component in the coagulation cascade. The membranes of activated platelets provide a surface for a number of critical reactions in the coagulation cascade, including the conversion of prothrombin to thrombin.

*secreted* from platelet granules. *ADP is particularly important in mediating platelet aggregation,* causing platelets to become "sticky" and adhere to one another (see below). Although strong agonists (such as thrombin and collagen) can trigger granule secretion even when aggregation is prevented, ADP can trigger granule secretion only in the presence of platelet aggregation. Presumably, this difference is caused by the set of intracellular effectors that are coupled to the various agonist receptors. Release of $Ca^{2+}$ ions is also important for the coagulation cascade, as discussed below.

## Platelet Aggregation and Consolidation

Both $TxA_2$ and ADP are potent mediators of platelet aggregation. $TxA_2$ promotes platelet aggregation through stimulation of G protein-coupled $TxA_2$ receptors in the platelet membrane (Fig. 21-4). Binding of $TxA_2$ to platelet $TxA_2$ receptors leads to activation of phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PI[4,5]P_2$) to yield inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ raises the cytosolic $Ca^{2+}$ concentration and DAG activates protein kinase C (PKC), which in turn promotes the activation of $PLA_2$. Through a poorly understood mechanism, $PLA_2$ activation induces the expression of functional GPIIb–IIIa, the membrane integrin that mediates platelet aggregation. ADP triggers platelet activation by binding to G protein-coupled ADP receptors on the platelet surface (Fig. 21-5). The two subtypes of G protein-coupled platelet ADP receptors are termed P2Y1 receptors and P2Y(ADP) receptors. P2Y1, a $G_q$-coupled receptor, releases intracellular calcium stores through activation of phospholipase C. P2Y(ADP), a $G_i$-coupled receptor, inhibits adenylyl cyclase. The P2Y(ADP) receptor is the target of the antiplatelet agents ticlopidine and clopidogrel (see below). Activation of ADP receptors mediates platelet shape change and expression of GPIIb–IIIa. Platelets aggregate with one another through a bridging molecule, **fibrinogen,** which has multiple binding sites for GPIIb–IIIa (Fig. 21-2). *Just as the vWF:GPIb interaction is important for platelet adhesion to exposed subendothelial collagen, the fibrinogen:GPIIb–IIIa interaction is critical for platelet aggregation.* Platelet aggregation ultimately leads to the formation of a reversible clot, or a **primary hemostatic plug.**

Activation of the **coagulation cascade** proceeds concurrently with the formation of the primary hemostatic plug, as described below. Activation of the coagulation cascade leads to the generation of fibrin, initially at the periphery of the primary hemostatic plug. Platelet pseudopods attach to the fibrin strands at the periphery of the plug and *contract.* Platelet contraction yields a compact, solid, irreversible clot, or a **secondary hemostatic plug.**

## SECONDARY HEMOSTASIS: THE COAGULATION CASCADE

Secondary hemostasis is also termed the **coagulation cascade.** The goal of this cascade is to form a stable fibrin clot at the site of vascular injury. Details of the coagulation cascade are presented schematically in Fig. 21-6. Several general principles should be noted.

First, the coagulation cascade is a sequence of enzymatic events. Most plasma coagulation factors circulate as inactive *proenzymes,* which are synthesized by the liver. These proenzymes become proteolytically cleaved by the activated factors that precede them in the cascade. The activation reaction is catalytic and not stoichiometric. For example, one "unit" of activated factor X can potentially generate 40 "units" of thrombin. This robust amplification process serves to generate large amounts of fibrin rapidly at a site of vascular injury.

Second, the major activation reactions in the cascade occur at sites where a **phospholipid-based protein–protein complex** has formed (Fig. 21-7). This complex is composed of a membrane surface (provided by activated platelets and activated endothelial cells), an enzyme (an activated coagulation factor), a substrate (the proenzyme form of the downstream coagulation factor), and a cofactor. The presence of negatively charged phospholipids, especially phosphatidylserine, is critical for assembly of the complex. Phosphatidylserine, which is normally sequestered in the inner leaflet of the plasma membrane, becomes redistributed to the outer leaflet following platelet or endothelial cell stimulation by agonists. Calcium is required for the enzyme, substrate, and cofactor to adopt the proper conformation for the proteolytic cleavage reaction of a coagulation factor proenzyme into its activated form.

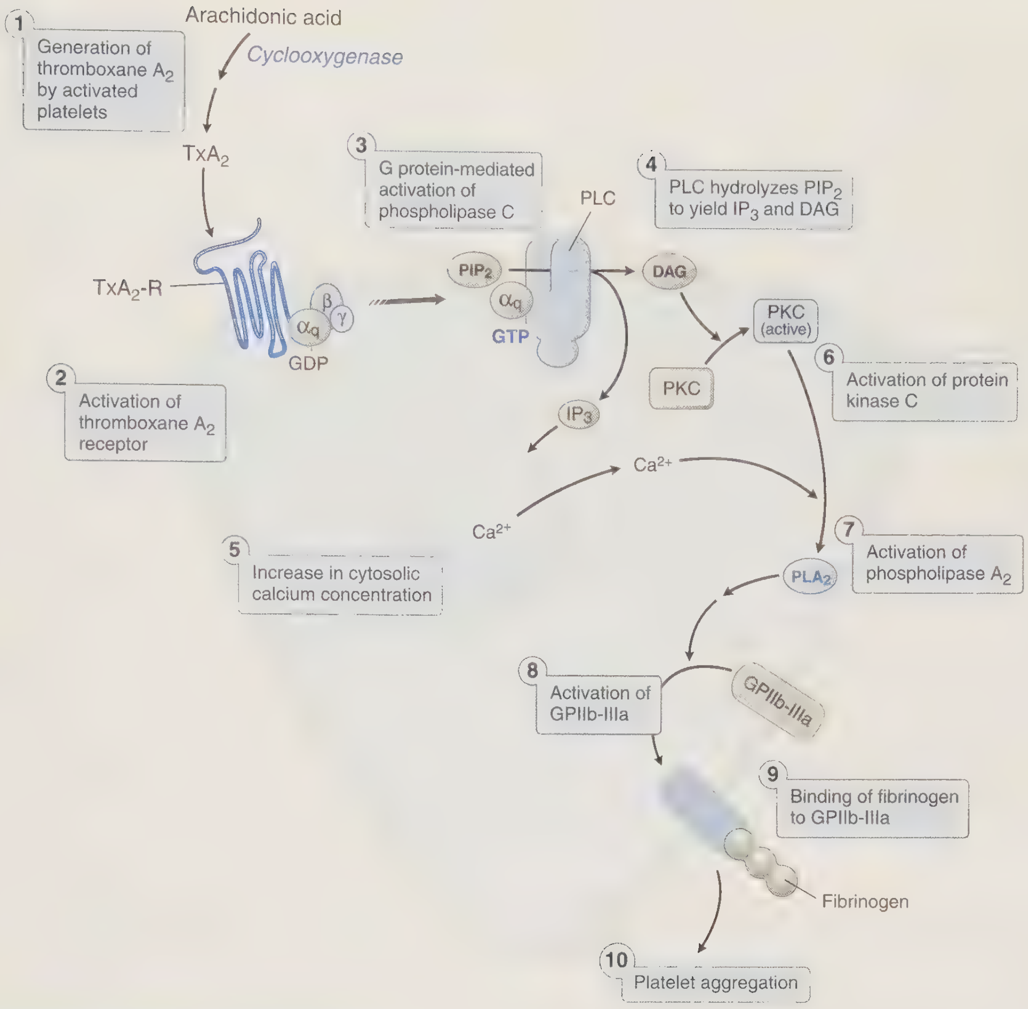

1
Generation of thromboxane A$_2$ by activated platelets
Arachidonic acid
Cyclooxygenase
TxA$_2$
TxA$_2$-R
β
γ
αq
GDP
2
Activation of thromboxane A$_2$ receptor
3
G protein-mediated activation of phospholipase C
PLC
PIP$_2$
αq
GTP
4
PLC hydrolyzes PIP$_2$ to yield IP$_3$ and DAG
DAG
PKC (active)
PKC
6
Activation of protein kinase C
IP$_3$
Ca$^{2+}$
Ca$^{2+}$
5
Increase in cytosolic calcium concentration
7
Activation of phospholipase A$_2$
PLA$_2$
8
Activation of GPIIb-IIIa
GPIIb-IIIa
9
Binding of fibrinogen to GPIIb-IIIa
Fibrinogen
10
Platelet aggregation

**Figure 21-4. Platelet Activation by Thromboxane A$_2$.** 1. Thromboxane A$_2$ (TxA$_2$) is generated from arachidonic acid in activated platelets; cyclooxygenase catalyzes the committed step in this process. 2. Secreted TxA$_2$ binds to the cell surface TxA$_2$ receptor (TxA$_2$-R), a G protein-coupled receptor. 3. The Gα isoform Gα$_q$ activates phospholipase C (PLC). 4. PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP$_2$) to yield inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG). 5. IP$_3$ raises the cytosolic free Ca$^{2+}$ concentration by promoting vesicular release of Ca$^{2+}$ into the cytosol. 6. DAG activates protein kinase C (PKC). 7. PKC activates phospholipase A$_2$ (PLA$_2$). 8. Through a poorly understood mechanism, activation of PLA$_2$ leads to the activation of GPIIb–IIIa. 9. Activated GPIIb–IIIa binds to fibrinogen. 10. Fibrinogen crosslinks platelets by binding to GPIIb–IIIa receptors present on other platelets. This crosslinking leads to platelet aggregation and formation of a primary hemostatic plug.

Third, the coagulation cascade has been divided traditionally into the **intrinsic and extrinsic pathways** (Fig. 21-6). This division is a result of in vitro testing and is essentially arbitrary. The intrinsic pathway is activated in vitro by factor XII (Hageman factor), while the extrinsic pathway is initiated in vivo by **tissue factor,** a lipoprotein expressed by activated endothelial cells, activated leukocytes, subendothelial smooth muscle cells, and subendothelial fibroblasts at the site of vascular injury. Although these two pathways converge at the activation of factor X, recent evidence shows that there also exists much interconnection between the two pathways. Because factor VII (activated by the extrinsic pathway) can proteolytically activate factor IX (a factor in the intrinsic pathway), the extrinsic pathway is now regarded as the primary pathway for the initiation of coagulation in vivo.

Finally, both the intrinsic and extrinsic coagulation pathways lead to the activation of factor X. In an important reaction that requires factor V, activated factor X proteolytically cleaves prothrombin (factor II) to **thrombin** (factor IIa) (Fig. 21-8). Thrombin is a multifunctional enzyme that acts in the coagulation cascade in two important ways: (1) it converts the soluble plasma protein fibrinogen into fibrin, which then forms long insoluble polymer fibers; and (2) it activates factor XIII, which cross links the fibrin polymers into a highly stable meshwork or clot. In addition, thrombin amplifies the clotting cascade by catalyzing the feedback activation of factors VIII and V. Thrombin also strongly activates platelets, causing granule release, platelet aggregation, and platelet-derived microvesicle generation. In addition to its procoagulant properties, thrombin acts to modulate the coagulation response. Thrombin binds to thrombin receptors on the *intact* vascular endothelial cells adjacent to the area of vascular injury and stimulates these cells to release the platelet inhibitors prostacyclin (PGI$_2$) and nitric oxide (NO), the profibrinolytic protein tissue-type plasminogen activator (t-PA), and the natural t-PA modulator plasminogen activator inhibitor type-1 (PAI-1) (see below).

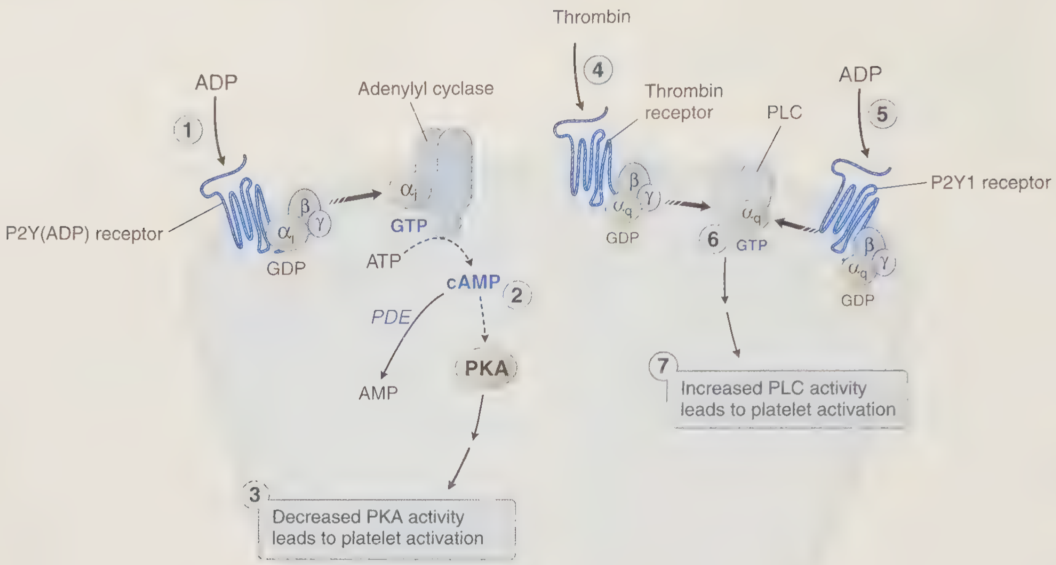


**Figure 21-5. Platelet Activation by ADP and Thrombin.** **Left panel:** 1. Binding of ADP to the P2Y(ADP) receptor activates a G protein, which inhibits adenylyl cyclase. 2. Inhibition of adenylyl cyclase decreases the synthesis of cAMP, and hence decreases protein kinase A (PKA) activation (dashed arrow). Any remaining cAMP is metabolized to AMP by phosphodiesterase (PDE). 3. PKA inhibits platelet activation through a series of poorly understood steps. Therefore, the decreased PKA activation that results from ADP binding to the P2Y(ADP) receptor causes platelet activation. **Right panel:** 4. Thrombin proteolytically cleaves the extracellular domain of its receptor. This cleavage creates a new N-terminus, which binds to an activation site on the thrombin receptor to activate a $G_q$ protein. 5. ADP also activates $G_q$ by binding to the P2Y1 receptor. 6. $G_q$ activation (by either thrombin or ADP) activates phospholipase C (PLC). 7. PLC activity leads to platelet activation, as shown in Figure 21-4. Note that ADP can activate platelets by binding to either the P2Y(ADP) receptor or the P2Y1 receptor.

The thrombin receptor, a protease-activated G protein-coupled receptor, is found in the plasma membrane of platelets, vascular endothelial cells, smooth muscle cells, and fibroblasts. Activation of the thrombin receptor involves proteolytic *cleavage* of the extracellular domain of the receptor by thrombin. The new $NH_2$-terminal-tethered ligand binds intramolecularly to a discrete site within the receptor and initiates intracellular signaling. Activation of the thrombin receptor results in G protein-mediated activation of PLC (Fig. 21-5) and inhibition of adenylyl cyclase.

## REGULATION OF HEMOSTASIS

Hemostasis is exquisitely regulated for two major reasons. First, hemostasis must be restricted to the local site of vascular injury. That is, activation of platelets and coagulation factors in the plasma should occur only at the site of endothelial damage, tissue factor expression, and procoagulant phospholipid exposure. Second, the size of the primary and secondary hemostatic plugs must be restricted so that the vascular lumen remains patent. After vascular injury, intact endothelium in the immediate vicinity of the injury becomes "activated." This activated endothelium presents a set of procoagulant factors that promote hemostasis at the site of injury, and anticoagulant factors that restrict propagation of the clot beyond the site of injury. The procoagulant factors, such as tissue factor and phosphatidylserine, tend to be *membrane-bound* and *localized* to the site of injury—these factors provide a surface on which the coagulation cascade can proceed. In contrast, the anticoagulant factors are generally *secreted* by the endothelium and are *soluble* in the blood. Thus, *the activated endothelium maintains a balance of procoagulant and anticoagulant factors to limit hemostasis to the site of vascular injury.*

After vascular injury, the endothelium surrounding the injured area participates in five separate mechanisms that limit the initiation and propagation of the hemostatic process to the immediate vicinity of the injury. These mechanisms involve prostacyclin ($PGI_2$), antithrombin III, proteins C and S, tissue factor pathway inhibitor (TFPI), and tissue-type plasminogen activator (t-PA).

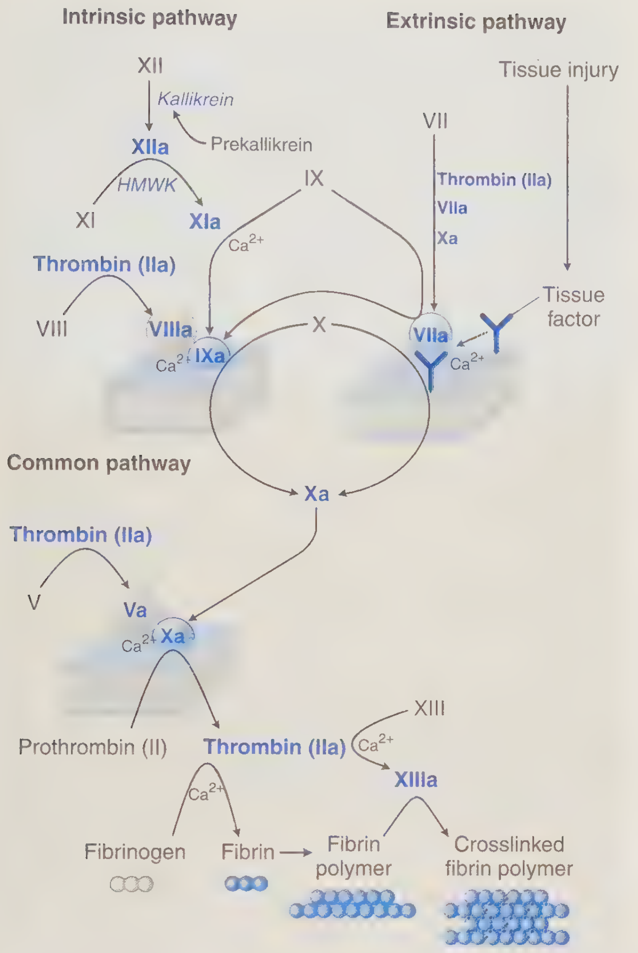

Intrinsic pathway
Extrinsic pathway
XII
Kallikrein
Prekallikrein
XIIa
HMWK
XI
XIa
IX
Ca2+
Thrombin (IIa)
VIII
VIIIa
Ca2+ IXa
Tissue injury
VII
Thrombin (IIa)
VIIa
Xa
X
VIIa
Ca2+
Tissue factor
Common pathway
Xa
Thrombin (IIa)
V
Va
Ca2+ Xa
Prothrombin (II)
Thrombin (IIa)
Ca2+
XIII
XIIIa
Ca2+
Fibrinogen
Fibrin
Fibrin polymer
Crosslinked fibrin polymer

**Figure 21-6. Coagulation Cascade.** The coagulation cascade is arbitrarily divided into the intrinsic pathway, the extrinsic pathway, and the common pathway. The intrinsic and extrinsic pathways converge at the level of factor X activation. The intrinsic pathway is largely an in vitro pathway, while the extrinsic pathway accounts for the majority of in vivo coagulation. The extrinsic pathway is initiated at sites of vascular injury by the expression of tissue factor on several different cell types, including activated endothelial cells, activated leukocytes, subendothelial vascular smooth muscle cells, and subendothelial fibroblasts. Note that $Ca^{2+}$ is a cofactor in many of the steps, and that a number of the steps occur on phospholipid surfaces provided by activated platelets, activated endothelial cells, and activated monocytes. Activated coagulation factors are shown in blue and indicated with a lower case "a." HMWK, high-molecular-weight kininogen.

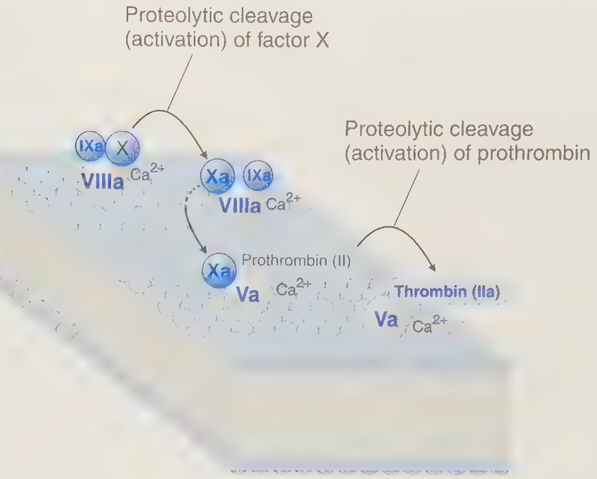

Proteolytic cleavage (activation) of factor X
IXa
X
VIIIa
Ca2+
Xa
IXa
VIIIa
Ca2+
Proteolytic cleavage (activation) of prothrombin
Xa
Prothrombin (II)
Va
Ca2+
Thrombin (IIa)
Va
Ca2+

**Figure 21-7. Coagulation Factor Activation on Phospholipid Surfaces.** Surface catalysis is critical for a number of the activation reactions in the coagulation cascade. Each activation reaction consists of an enzyme (e.g., factor IXa), a substrate (e.g., factor X), and a cofactor or reaction accelerator (e.g., factor VIIIa), all of which are assembled on the phospholipid surface of activated platelets and endothelial cells. $Ca^{2+}$ allows the enzyme and substrate to adopt the proper conformation in each activation reaction. In the example shown, factor VIIIa and $Ca^{2+}$ act as cofactors in the factor IXa-mediated cleavage of factor X to factor Xa. Factor Va and $Ca^{2+}$ then act as cofactors in the factor Xa-mediated cleavage of prothrombin to thrombin.

**Prostacyclin ($PGI_2$)** is an eicosanoid (i.e., a metabolite of arachidonic acid) that is synthesized and secreted by the endothelium. By acting through $G_s$ protein-coupled platelet surface $PGI_2$ receptors, this metabolite increases cAMP levels within platelets and, thereby, inhibits platelet aggregation and platelet granule release. $PGI_2$ also has potent vasodilatory effects; this mediator induces vascular smooth muscle relaxation by increasing cAMP levels within the vascular smooth muscle cells. (Note that these mechanisms are physiologically antagonistic to those of $TxA_2$, which induces platelet activation and vasoconstriction by decreasing intracellular cAMP levels.) Both of these actions of $PGI_2$ prevent platelets from adhering to the intact endothelium that surrounds the site of vascular injury and maintain vascular patency around the site of injury.

**Antithrombin III** inactivates thrombin and other coagulation factors (IXa, Xa, XIa, and XIIa, where "a" denotes an "activated" factor) by forming a stoichiometric complex with the coagulation factor (Fig. 21-9). These interactions are enhanced by a heparin-like molecule that is expressed at the surface of intact endothelial cells, ensuring that this mechanism is operative at all locations in the vascular tree *except* where endothelium is denuded at the site of vascular injury. (These endothelial cell surface proteoglycans are referred to as "heparin-like" because they are the physiologic equivalent of the pharmacologic agent heparin, discussed below.) Heparin-like molecules on the endothelial cells bind to and activate antithrombin III, which is then primed to complex with (and, thereby, inactivate) the activated coagulation factors.

**Protein C and protein S** are vitamin K-dependent proteins that slow the coagulation cascade by inactivating coagulation factors Va and VIIIa. Protein C and protein S are part of a feedback control mechanism, in which excess thrombin generation leads to activation of protein C which, in turn, helps to prevent the enlarging fibrin clot from occluding the vascular lumen. Specifically, the endothelial cell surface protein **thrombomodulin** is a receptor for both thrombin and protein C in the plasma. Thrombomodulin binds these proteins in a conformation so that thrombomodulin-bound thrombin is set up to cleave protein C to activated protein C (also known as protein Ca). In a reaction that requires the cofactor protein S, activated protein C then inhibits clotting by proteolytically inactivating factors Va and VIIIa.

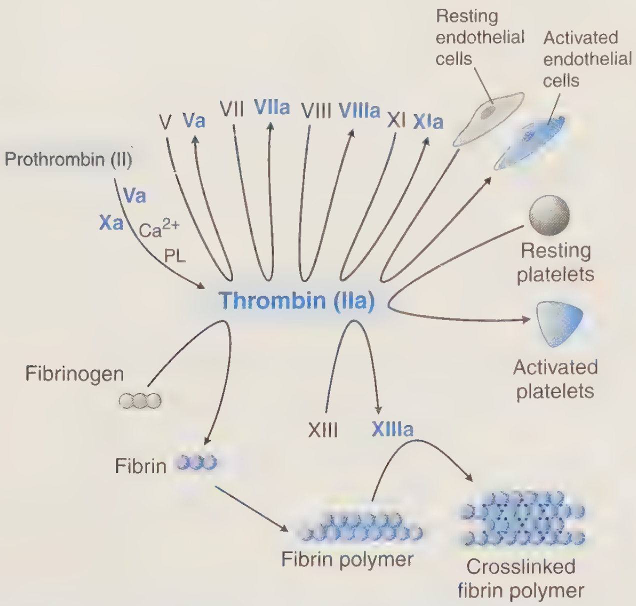

Resting endothelial cells
Activated endothelial cells
V Va
VII VIIa
VIII VIIIa
XI XIa
Prothrombin (II)
Va
Xa
$Ca^{2+}$
PL
Thrombin (IIa)
Resting platelets
Activated platelets
Fibrinogen
XIII
XIIIa
Fibrin
Fibrin polymer
Crosslinked fibrin polymer

**Figure 21-8. Central Role of Thrombin in the Coagulation Cascade.** In the coagulation cascade, prothrombin is cleaved to thrombin by factor Xa; factor Va and $Ca^{2+}$ act as cofactors in this reaction, and the reaction takes place on an activated (phosphatidylserine-expressing) phospholipid surface (PL). Thrombin converts the soluble plasma protein fibrinogen to fibrin, which spontaneously polymerizes. Thrombin also activates factor XIII, a transglutaminase that crosslinks the fibrin polymers into a highly stable meshwork or clot. Thrombin also activates cofactors V and VIII, as well as coagulation factors VII and XI. In addition, thrombin activates both platelets and endothelial cells. Finally, thrombin stimulates the release of several antithrombotic factors—including $PGI_2$, NO, and t-PA—from resting (intact) endothelial cells near the site of vascular injury; these factors limit primary and secondary hemostasis to the injured site (not shown).

**Tissue factor pathway inhibitor (TFPI),** as its name suggests, limits the action of tissue factor (TF). The coagulation cascade is initiated when factor VIIa complexes with TF at the site of vascular injury (Fig. 21-6). The resulting VIIa:TF complex catalyzes the activation of factors IX and X. After limited quantities of factors IXa and Xa are generated, the VIIa:TF complex becomes feedback inhibited by TFPI in a two-step reaction. First, TFPI binds to factor Xa and neutralizes its activity in a $Ca^{2+}$-independent reaction. Subsequently, the TFPI:Xa complex interacts with the VIIa:TF complex via a second domain on TFPI, so that a quaternary Xa:TFPI:VIIa:TF complex is formed. The molecular "knots" of the TFPI molecule hold together the quaternary complex tightly and completely inactivate the VIIa:TF complex. In this manner, TFPI prevents excessive TF-mediated activation of factors IX and X.

**Plasmin** exerts its anticoagulant effect by proteolytically cleaving fibrin into fibrin degradation products. Because plasmin has powerful antithrombotic effects, the *formation* of plasmin has intrigued researchers for many years, and a number of pharmacologic agents have been developed to target the plasmin formation pathway (Fig. 21-10). Plasmin is generated by the proteolytic cleavage of plasminogen, a plasma protein that is synthesized in the liver. The proteolytic cleavage is catalyzed by **tissue-type plasminogen activator (t-PA),** which is synthesized and secreted by the endothelium. Plasmin activity is carefully modulated by three regulatory mechanisms, so that plasmin action is restricted to the site of clot formation. First, t-PA is most effective when it is bound to a fibrin meshwork. Second, t-PA activity can be inhibited by **plasminogen activator inhibitor (PAI).** When local concentrations of thrombin and inflammatory cytokines (such as IL-1 and TNF-α) are *high,* endothelial cells *increase* the release of PAI, preventing t-PA from activating plasmin. This

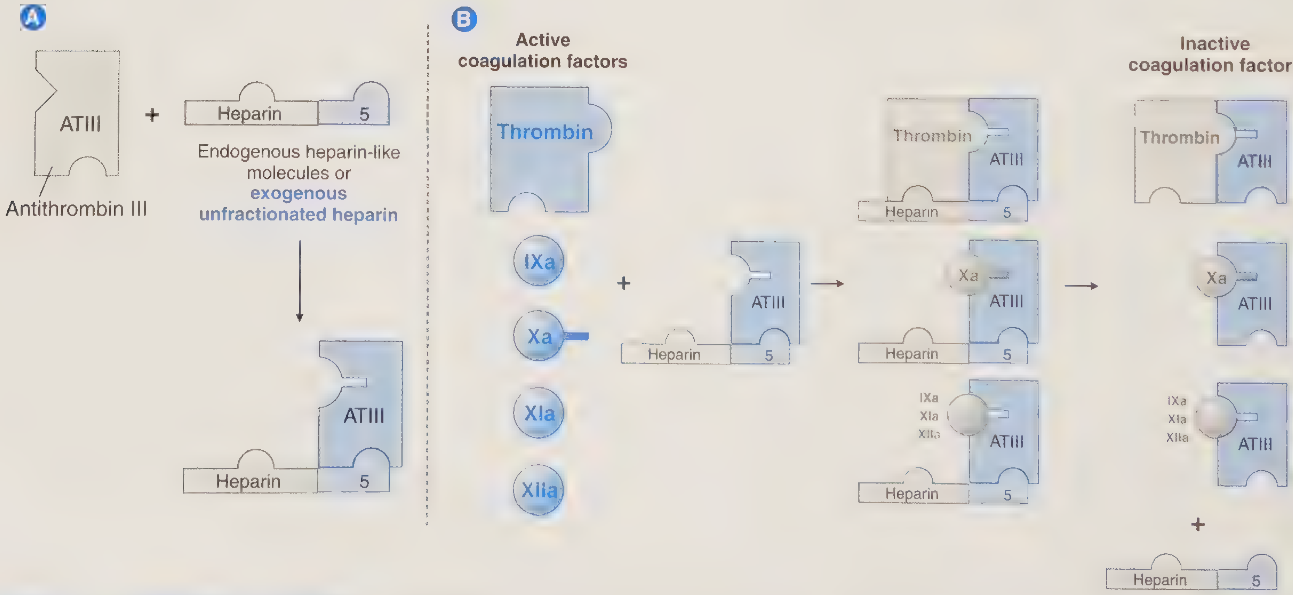

A
ATIII
+
Heparin
5
Antithrombin III
Endogenous heparin-like molecules or
exogenous unfractionated heparin
ATIII
Heparin
5
B
Active coagulation factors
Thrombin
IXa
Xa
XIa
XIIa
+
ATIII
Heparin
5
Thrombin
ATIII
Heparin
5
Xa
ATIII
Heparin
5
IXa
XIa
XIIa
ATIII
Heparin
5
Inactive coagulation factors
Thrombin
ATIII
Xa
ATIII
IXa
XIa
XIIa
ATIII
+
Heparin
5

**Figure 21-9. Antithrombin III Action.** Antithrombin III (ATIII) inactivates thrombin and factors IXa, Xa, XIa, and XIIa by forming a stoichiometric complex with these coagulation factors. These reactions are catalyzed physiologically by heparin-like molecules expressed on healthy endothelial cells; sites of vascular injury do not express heparin-like molecules because the endothelium is denuded or damaged. Pharmacologically, these reactions are catalyzed by exogenously administered heparin. In more detail, the binding of heparin to ATIII induces a conformational change in ATIII **(A)** that allows the ATIII to bind thrombin or coagulation factors IXa, Xa, XIa or XIIa. The stoichiometric complex between ATIII and the coagulation factor is highly stable, allowing heparin to dissociate without breaking up the complex. In this way, heparin serves as a true catalyst for the inactivation reaction **(B).**

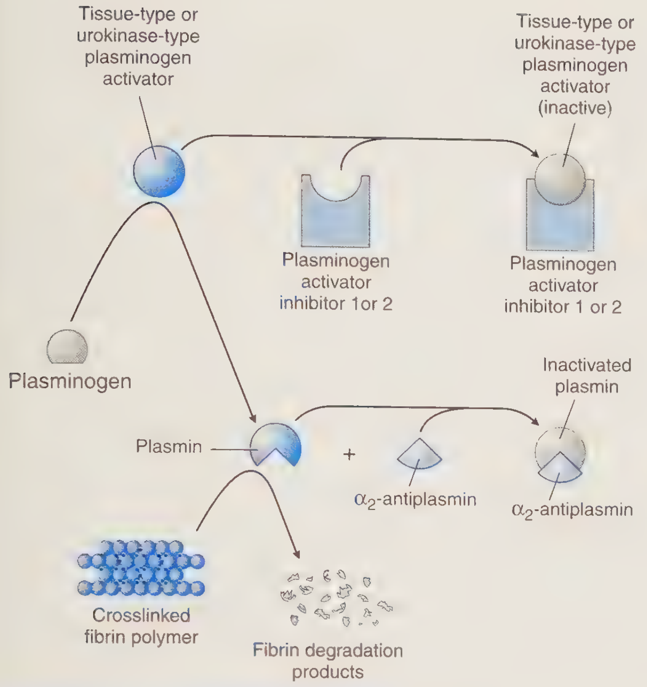

Tissue-type or urokinase-type plasminogen activator
Tissue-type or urokinase-type plasminogen activator (inactive)
Plasminogen activator inhibitor 1or 2
Plasminogen activator inhibitor 1 or 2
Plasminogen
Inactivated plasmin
Plasmin
+
$\alpha_2$-antiplasmin
$\alpha_2$-antiplasmin
Crosslinked fibrin polymer
Fibrin degradation products

**Figure 21-10. The Fibrinolytic System.** Plasmin is formed by the proteolytic cleavage of plasminogen by tissue-type or urokinase-type plasminogen activator. Plasmin formation can be inhibited by plasminogen activator inhibitor 1 or 2, which binds to and inactivates plasminogen activators. In the fibrinolytic reaction, plasmin cleaves crosslinked fibrin polymers into fibrin degradation products. $\alpha_2$-Antiplasmin, which circulates in the bloodstream, neutralizes free plasmin in the circulation.

ensures that a stable fibrin clot forms at the site of vascular injury. Last, **$\alpha_2$-antiplasmin** is a plasma protein that neutralizes free plasmin in the circulation and, thereby, prevents random degradation of plasma fibrinogen. Plasma fibrinogen is important for platelet aggregation in primary hemostasis (see above), and it is also the precursor for the fibrin polymer that is required to form a stable clot.

## PATHOGENESIS OF THROMBOSIS

Thrombosis is the pathologic extension of hemostasis. In thrombosis, coagulation reactions are inappropriately regulated so that a clot uncontrollably enlarges and occludes the lumen of a blood vessel. The pathologic clot is now termed a **thrombus.** Three major factors predispose to thrombus formation—endothelial injury, abnormal blood flow, and hypercoagulability. These three factors influence one another, and are collectively known as **Virchow's triad** (Fig. 21-11).

### ENDOTHELIAL INJURY

Endothelial injury is the most dominant influence on thrombus formation in the *heart* and the *arterial circulation.* There are many possible causes of endothelial injury, including changes in shear stress associated with hypertension or turbulent flow, hyperlipidemia, elevated blood glucose in diabetes mellitus, traumatic vascular injury, and some infections. (Recall that Mr. Soprano developed coronary artery thrombosis, which was probably attributable to endothelial injury secondary to hypertension and cigarette smoking.)

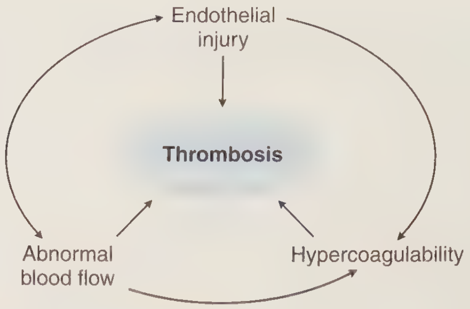


**Figure 21-11. Virchow's Triad.** Endothelial injury, abnormal blood flow, and hypercoagulability are three factors that predispose to thrombus formation. These three factors are interrelated; endothelial injury predisposes to abnormal blood flow and hypercoagulability, while abnormal blood flow can cause both endothelial injury and hypercoagulability.

Endothelial injury predisposes the vascular lumen to thrombus formation through three mechanisms. First, platelet activators, such as exposed subendothelial collagen, promote platelet adherence to the injured site. Second, exposure of tissue factor on injured endothelium initiates the coagulation cascade. Third, natural antithrombotics, such as t-PA and $PGI_2$, become depleted at the site of vascular injury because these mechanisms rely on the functioning of an intact endothelial cell layer.

### ABNORMAL BLOOD FLOW

Abnormal blood flow refers to a state of **turbulence** or **stasis** rather than laminar flow. Atherosclerotic plaques commonly predispose to local, turbulent blood flow. Bifurcations of blood vessels can also create areas of turbulent flow. Turbulent blood flow causes endothelial injury, forms countercurrents, and creates local pockets of stasis. Local stasis can also result from formation of an aneurysm (a focal out-pouching of a vessel or a cardiac chamber) and from myocardial infarction. In the latter condition, a region of noncontractile (infarcted) myocardium serves as a favored site for stasis. Cardiac arrhythmias, such as atrial fibrillation, can also generate areas of local stasis. Stasis is the major impetus for the formation of *venous* thrombi.

Disruption of normal blood flow by turbulence or stasis promotes thrombosis by three major mechanisms. First, the absence of laminar blood flow allows platelets to come into close proximity to the vessel wall. Second, stasis inhibits the flow of fresh blood into the vascular bed, so that activated coagulation factors in the region are not diluted by fresh blood. Finally, abnormal blood flow promotes endothelial cell activation, which leads to a prothrombotic state.

### HYPERCOAGULABILITY

Hypercoagulability is generally less important than endothelial injury and abnormal blood flow in predisposing to thrombosis, but this condition can be an important factor in some

**TABLE 21-1** Common Causes of Hypercoagulability

| CONDITION | MECHANISM OF HYPERCOAGULABILITY |
|---|---|
| **Primary (Genetic)** | |
| Factor V Leiden | Resistance to activated protein C → excess factor Va |
| Protein C or S deficiency | Decreased proteolytic inactivation of factors VIIIa and Va |
| Hyperhomocysteinemia | Endothelial damage due to accumulation of homocysteine |
| Antithrombin deficiency | Decreased inactivation of factors IIa, IXa, and Xa |
| **Secondary (Acquired: Disease or Drug-Induced)** | |
| Surgery/Trauma | Venous stasis, immobilization, tissue injury |
| Postpartum period | Venous stasis, increased coagulation factors, tissue trauma |
| Malignancy | Tumor cell induction of tissue factor expression |
| Antiphospholipid syndrome | Autoantibodies to negatively charged phospholipids → ↑ platelet adhesion |
| Nephrotic syndrome | Loss of antithrombin in urine, ↑ fibrinogen, ↑ platelet activation |
| Myeloproliferative syndromes | Elevated blood viscosity, altered platelets |
| Paroxysmal nocturnal hemoglobinuria | Unknown, possibly leaky platelets |
| Heparin-induced thrombocytopenia | Antibodies to platelet factor 4 → platelet activation |
| Oral contraceptive pills, estrogen replacement therapy | ↑ Hepatic synthesis of coagulation factors and/or effects of estrogen on endothelium (effect may be more prominent in patients with underlying primary hypercoagulability) |

patients. Hypercoagulability refers to an abnormally heightened coagulation response to vascular injury, resulting from either: (1) **primary (genetic) disorders;** or (2) **secondary (acquired) disorders** (see Table 21-1). (Hypocoagulable states, or **hemorrhagic disorders,** can also result from primary or secondary causes; see Box 21-1 for an example.)

Among the genetic causes of hypercoagulability, the most prevalent known mutation resides in the gene for coagulation factor V. It is estimated that 2%–15% of the Caucasian population in the U.S. carries mutations in the factor V gene. The most common mutation is the Leiden mutation, in which glutamine is substituted for arginine at position 506. This position

## BOX 21-1. Hemorrhagic Disorders: The Case of Hemophilia A

When the vascular endothelium is injured, the hemostatic process ensures localized, stable clot formation without obstruction of the vascular lumen. Just as thrombosis constitutes a pathologic variation on this otherwise orchestrated physiologic process, disorders involving insufficient amounts of functional platelets or coagulation factors can lead to a hypocoagulable state characterized clinically by episodes of uncontrolled hemorrhage. Hemorrhagic disorders result from a multitude of causes, including disorders of the vasculature, vitamin K deficiency, and disorders or deficiencies of platelets, coagulation factors, and von Willebrand factor. Hemophilia A serves as an example of a hemorrhagic disorder in which hypocoagulability is the underlying pathology.

Hemophilia A is the most common genetic disorder of serious bleeding. The hallmark of the disorder is a reduction in the amount or activity of coagulation factor VIII. The syndrome has an X-linked mode of transmission, and the majority of patients are males or homozygous females. Thirty percent of patients have no family history of hemophilia A, and presumably represent spontaneous mutations. The severity of the disease depends on the type of mutation in the factor VIII gene. Patients with 6%–50% of normal factor VIII activity manifest a mild form of the disease; those with 2%–5% activity manifest moderate disease; patients with less than 1% activity develop severe disease. All symptomatic patients demonstrate easy bruisability and can develop massive hemorrhage after trauma or surgery. Spontaneous hemorrhage can occur in body areas that are normally subjected to minor trauma, including joint spaces, where spontaneous hemorrhage leads to the formation of hemarthroses. Petechiae (microhemorrhages involving capillaries and small vessels, especially in mucocutaneous areas), which are usually an indication of platelet disorders, are absent in patients with hemophilia.

Patients with hemophilia A are currently treated with infusions of factor VIII that is either recombinant or derived from human plasma. Factor VIII infusion therapy is sometimes complicated in patients who develop antibodies against factor VIII. HIV infection was a serious complication of infusion therapy in patients who received factor VIII products before routine screening of blood for HIV infection was instituted (before the mid-1980s). Some sources suggest that the entire cohort of hemophiliacs who received factor VIII concentrates (factor VIII concentrated from the blood of many individuals) between 1981 to 1985 have been infected with HIV. With current blood screening practices and the development of recombinant factor VIII, the risk of contracting HIV through factor VIII infusions is now virtually zero.

is important because it is part of a site in factor Va that is marked for proteolytic cleavage by activated protein C. The mutant factor V Leiden protein is resistant to proteolytic cleavage by activated protein C. As a result of the Leiden mutation, factor Va is allowed to accumulate and, thereby, to promote coagulation. Other genetic disorders that predispose some individuals to thrombosis include mutations in the fibrinogen, protein C, protein S, and antithrombin III genes. Although the latter disorders are relatively uncommon, patients with a genetic deficiency of protein C, protein S, or antithrombin III often present with spontaneous venous thrombosis.

Hypercoagulability can sometimes be acquired (secondary) rather than genetic. An example of acquired hypercoagulability is the heparin-induced thrombocytopenia syndrome. In some patients, administration of the anticoagulant heparin stimulates the immune system to generate circulating antibodies directed against a complex consisting of heparin and platelet factor 4. Because platelet factor 4 is present on platelet and endothelial cell surfaces, antibody binding to the heparin: platelet factor 4 complex results in antibody-mediated removal of platelets from the circulation; that is, in thrombocytopenia. In some patients, however, antibody binding also causes platelet activation, endothelial injury, and a prothrombotic state. Although both unfractionated and low molecular weight heparin (see below) can cause thrombocytopenia, researchers have recently found that low molecular weight heparin has a lower incidence of thrombocytopenia than does unfractionated heparin.

# PHARMACOLOGIC CLASSES AND AGENTS

Drugs have been developed to prevent and/or reverse thrombus formation. These drugs fall into three classes: antiplatelet agents, anticoagulants, and thrombolytic agents.

## ANTIPLATELET AGENTS

Because formation of a localized platelet plug in response to endothelial injury is usually the initial step in arterial thrombosis, inhibition of platelet function is a useful prophylactic and therapeutic strategy against myocardial infarction and stroke caused by thrombosis in coronary and cerebral arteries, respectively. The classes of antiplatelet agents in clinical use currently include cyclooxygenase inhibitors, phosphodiesterase inhibitors, ADP receptor pathway inhibitors, and GPIIb–IIIa antagonists.

### Cyclooxygenase Inhibitors

By inhibiting the synthesis of various prostaglandins, **aspirin** and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the platelet release reaction and interfere with normal platelet aggregation.

The biochemistry of prostaglandin synthesis in platelets and endothelial cells provides a basis for understanding the mechanism of action of aspirin as an antiplatelet agent. Figure 21-12 depicts schematically the prostaglandin synthesis pathway, which is discussed in more detail in Chapter 40, Pharmacology of Eicosanoids. Briefly, activation of both platelets and endothelial cells induces phospholipase $A_2$ ($PLA_2$) to cleave membrane phospholipids and release arachidonic acid. Arachidonic acid is then transformed into a cyclic endoperoxide (also known as prostaglandin $G_2$ or $PGG_2$) by the enzyme cyclooxygenase (COX). In platelets, the cyclic endoperoxide is converted into a series of prostaglandins that culminates in the synthesis of thromboxane $A_2$ ($TxA_2$). Acting through the cell surface $TxA_2$ receptor, $TxA_2$ causes localized vasoconstriction and is a potent inducer of platelet aggregation and the platelet release reaction. In endothelial cells, the cyclic endoperoxide is converted into a series of prostaglandins that culminates in the synthesis of prostacyclin ($PGI_2$). $PGI_2$, in turn, causes localized vasodilation and inhibits platelet aggregation and the platelet release reaction.

Aspirin acts by acetylating *covalently* a serine residue near the active site of the COX enzyme, thereby inhibiting the synthesis of the cyclic endoperoxide and the various metabolites of the cyclic endoperoxide. In the absence of $TxA_2$, there is a marked decrease in platelet aggregation and the platelet release reaction (Fig. 21-13A). *Because platelets do not contain DNA or RNA, these cells cannot regenerate new COX enzyme once aspirin has permanently inactivated all of the available COX enzyme.* That is, the platelets become irreversibly "poisoned" for the lifetime of these cells (7–10 days). Although aspirin also inhibits the COX enzyme in endothelial cells, its action is not permanent in endothelial cells because these cells are able to synthesize new COX molecules. Thus, the endothelial cell production of prostacyclin is relatively unaffected by aspirin at pharmacologically low doses (see below).

Aspirin is most often used as an antiplatelet agent to prevent arterial thrombosis leading to stroke, transient ischemic attack, and myocardial infarction. Because the action of aspirin on platelets is permanent, it is most effective as a selective antiplatelet agent when taken in *low doses and/or at infrequent intervals*. For example, aspirin is often used as an antiplatelet agent at a dose of 81 mg once daily, while a typical anti-inflammatory dose of this agent could be 650 mg three to four times daily. Taken at high doses, aspirin can inhibit prostacyclin production without increasing the effectiveness of the drug as an antiplatelet agent. A more extended discussion of the uses and toxicities of aspirin is found in Chapter 40. Compared with aspirin, other NSAIDs are not as widely used in the prevention of arterial thrombosis because the inhibitory action of these drugs on cyclooxygenase is not permanent.

COX-1 is the predominant COX isoform in platelets and endothelial cells. Because aspirin inhibits COX-1 and COX-2 nonselectively, this drug serves as an effective antiplatelet agent. In contrast, the newer selective COX-2 inhibitors cannot be used as antiplatelet agents. However, when used as anti-inflammatory agents, the COX-2 inhibitors also do not have the unwanted antiplatelet side effects that often accompany aspirin use (see Chapter 40).

### Phosphodiesterase Inhibitors

In platelets, an *increase* in the concentration of intracellular cAMP leads to a *decrease* in platelet aggregability. Platelet cAMP levels are regulated physiologically by $TxA_2$ and $PGI_2$, among other mediators (see above). The mechanism by which increased intracellular cAMP concentration leads to decreased platelet aggregability is not well understood. cAMP activates protein kinase A, which, in turn, through several incompletely

*(text continues on page 348)*

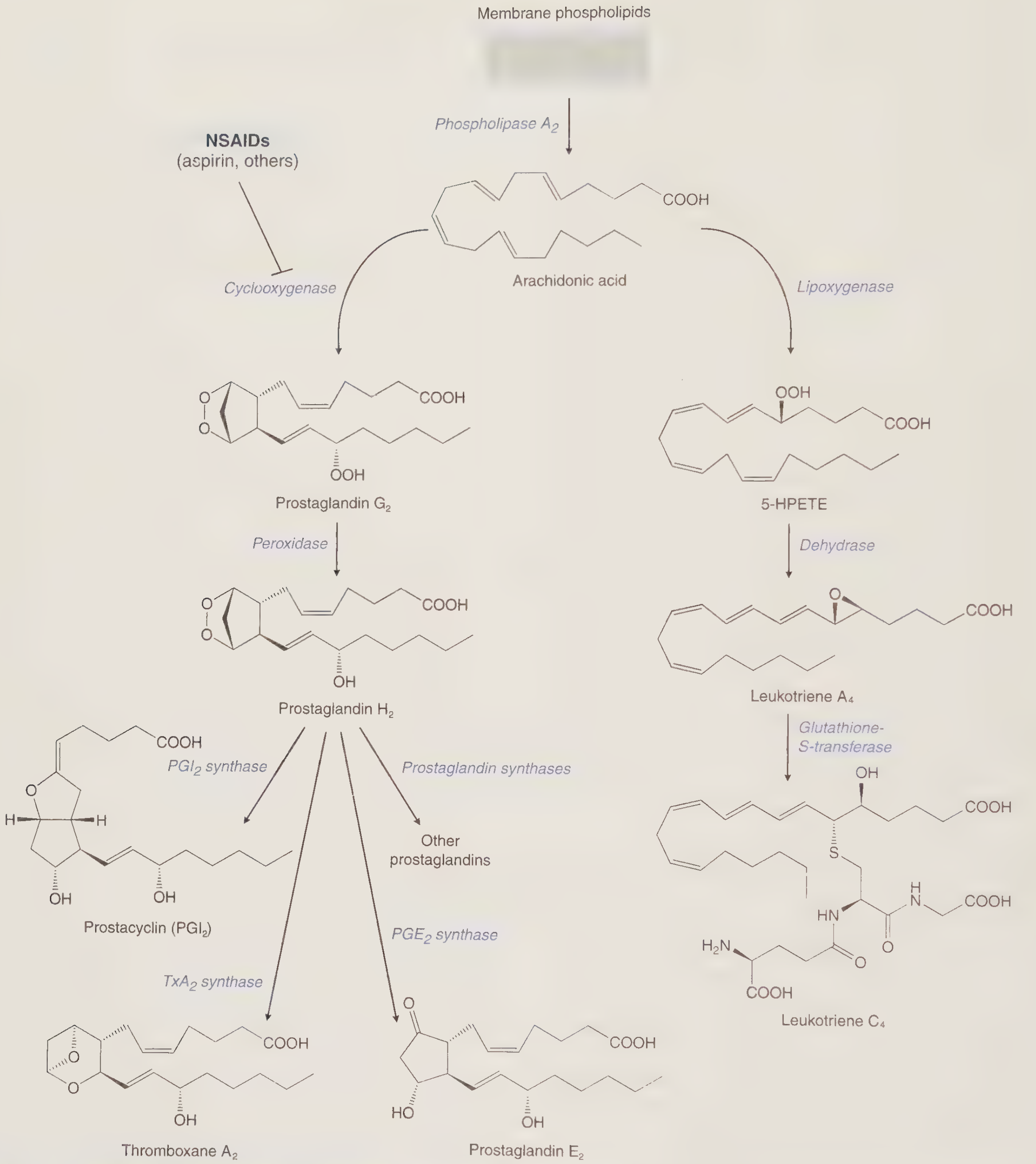

Membrane phospholipids
Phospholipase $A_2$
NSAIDs
(aspirin, others)
Cyclooxygenase
COOH
Arachidonic acid
Lipoxygenase
O
O
COOH
OOH
Prostaglandin $G_2$
OOH
COOH
5-HPETE
Peroxidase
Dehydrase
O
O
COOH
OH
Prostaglandin $H_2$
O
COOH
Leukotriene $A_4$
COOH
PGI$_2$ synthase
Prostaglandin synthases
Glutathione-
S-transferase
O
H
H
OH
OH
Prostacyclin (PGI$_2$)
Other
prostaglandins
OH
COOH
S
H
N
COOH
HN
O
$H_2N$
O
COOH
Leukotriene $C_4$
PGE$_2$ synthase
TxA$_2$ synthase
O
O
O
COOH
OH
Thromboxane $A_2$
COOH
HO
OH
Prostaglandin $E_2$

**Figure 21-12. Overview of Prostaglandin Synthesis.** Membrane phospholipids are cleaved by phospholipase $A_2$ to release free arachidonic acid. Arachidonic acid can be metabolized through either of two major pathways, the cyclooxygenase pathway or the lipoxygenase pathway. The cyclooxygenase pathway, which is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), converts arachidonic acid into prostaglandins and thromboxanes. Platelets express TxA$_2$ synthase and synthesize the pro-aggregatory mediator thromboxane $A_2$; endothelial cells express PGI$_2$ synthase and synthesize the anti-aggregatory mediator prostacyclin. The lipoxygenase pathway converts arachidonic acid into leukotrienes, which are potent inflammatory mediators. (See Chapter 40, Pharmacology of Eicosanoids, for a detailed discussion of the lipoxygenase and cyclooxygenase pathways.) Aspirin inhibits cyclooxygenase by covalent acetylation of the enzyme near its active site. Because platelets lack the capability to synthesize new proteins, aspirin inhibits thromboxane synthesis for the life of the platelet.

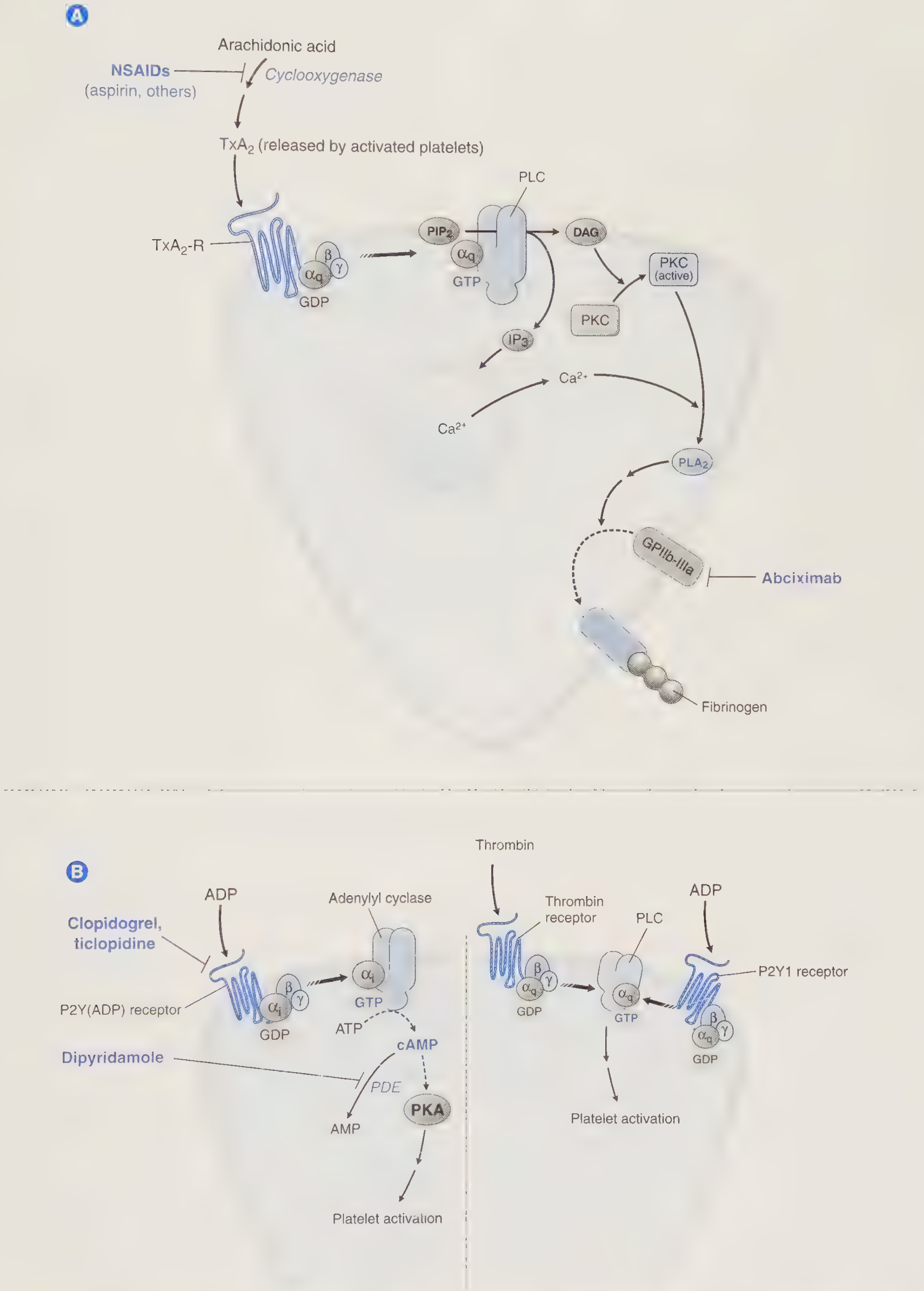

A
Arachidonic acid
NSAIDs
(aspirin, others)
Cyclooxygenase
TxA2 (released by activated platelets)
TxA2-R
β
γ
αq
GDP
PLC
PIP2
αq
GTP
DAG
PKC (active)
PKC
IP3
Ca2+
Ca2+
PLA2
GPIIb-IIIa
Abciximab
Fibrinogen
B
ADP
Clopidogrel, ticlopidine
P2Y(ADP) receptor
β
γ
αi
GDP
Adenylyl cyclase
αi
GTP
ATP
cAMP
Dipyridamole
PDE
PKA
AMP
Platelet activation
Thrombin
Thrombin receptor
β
γ
αq
GDP
PLC
αq
GTP
ADP
P2Y1 receptor
β
γ
αq
GDP
Platelet activation

**Figure 21-13. Mechanism of Action of Antiplatelet Agents.** **A,** Nonsteroidal anti-inflammatory drugs (NSAIDs) and abciximab inhibit steps in thromboxane $A_2$ ($TxA_2$)-mediated platelet activation. Aspirin inhibits cyclooxygenase by covalent acetylation of the enzyme near its active site, leading to decreased $TxA_2$ production. The effect is profound because platelets lack the ability to synthesize new enzyme molecules. Monoclonal antibody GPIIb–IIIa inhibitors, such as abciximab, inhibit platelet aggregation by preventing activation of GpIIb–IIIa (dashed line), leading to decreased platelet crosslinking by fibrinogen. **B,** Clopidogrel, ticlopidine, and dipyridamole inhibit steps in ADP-mediated platelet activation. Clopidogrel and ticlopidine are antagonists of the P2Y(ADP) receptor. Dipyridamole inhibits phosphodiesterase (PDE), thereby preventing the breakdown of cAMP and increasing cytoplasmic cAMP concentrations.

elucidated mechanisms, decreases the availability of intracellular $Ca^{2+}$ necessary for platelet aggregation (Fig. 21-13B). Inhibitors of platelet phosphodiesterase decrease platelet aggregability by inhibiting cAMP degradation, while activators of platelet adenylyl cyclase decrease platelet aggregability by increasing cAMP synthesis. (There are currently no direct adenylyl cyclase activators in clinical use.)

**Dipyridamole** is an inhibitor of platelet phosphodiesterase that decreases platelet aggregability (Fig. 21-13B). Dipyridamole by itself has only weak antiplatelet effects, and is, therefore, usually administered in combination with warfarin or aspirin. The combination of dipyridamole and warfarin can be used to inhibit embolization from prosthetic heart valves, while the combination of dipyridamole and aspirin can be used to reduce the likelihood of thrombosis in patients with a thrombotic diathesis. Dipyridamole also has vasodilatory properties. It may paradoxically induce angina in patients with coronary artery disease by causing the coronary steal phenomenon, which involves intense dilation of coronary arterioles (see Chapter 20, Pharmacology of Vascular Tone).

## ADP Receptor Pathway Inhibitors

Both **ticlopidine** and **clopidogrel** are derivatives of thienopyridine. These agents, which irreversibly inhibit the ADP-dependent pathway of platelet activation, have antiplatelet effects in vitro and in vivo. Ticlopidine and clopidogrel are thought to act by covalently modifying and inactivating the platelet P2Y(ADP) receptor, which is physiologically coupled to the inhibition of adenylyl cyclase (Fig. 21-13B). The use of ticlopidine is mainly restricted to the treatment of acute cerebral ischemia, while the better tolerated clopidogrel is administered to patients with atherosclerotic vascular disease.

## GPIIb–IIIa Antagonists

As noted above, platelet membrane GPIIb–IIIa receptors are important because they constitute the final common pathway of platelet aggregation, serving to bind fibrinogen molecules that bridge platelets to one another. A variety of stimuli (e.g., $TxA_2$, ADP, epinephrine, collagen, and thrombin), acting through diverse signaling molecules, are capable of inducing the expression of GPIIb–IIIa on the platelet surface. It could, therefore, be predicted that antagonists of GPIIb–IIIa would prevent fibrinogen binding to the GPIIb–IIIa receptor and, thus, inhibit platelet aggregation. **Eptifibatide,** the GPIIb–IIIa receptor antagonist used in the opening case, is a highly efficacious inhibitor of platelet aggregation. Eptifibatide, a synthetic peptide, antagonizes the platelet GPIIb–IIIa receptor with high affinity. It has been used to reduce ischemic events in patients undergoing percutaneous coronary angioplasty, and to treat unstable angina and non–Q-wave myocardial infarction.

**Abciximab** is a chimeric mouse–human monoclonal antibody directed against the human GPIIb–IIIa receptor. Experiments in vitro have shown that occupation of 50% of platelet GPIIb–IIIa receptors by abciximab significantly reduces platelet aggregation. The binding of abciximab to GPIIb–IIIa is essentially *irreversible*, with a dissociation half-time of 18–24 hours. Clinical trials have demonstrated that adding abciximab to conventional antithrombotic therapy reduces both long-term and short-term ischemic events in patients undergoing high-risk coronary angioplasty.

**Tirofiban** is a nonpeptide tyrosine analogue that reversibly antagonizes fibrinogen binding to the platelet GPIIb–IIIa receptor. Both in vitro and in vivo studies have demonstrated the ability of tirofiban to inhibit platelet aggregation. Tirofiban has been approved for use in patients with acute coronary syndromes.

Because of their mechanism of action as antiplatelet agents, all of the GPIIb–IIIa antagonists can cause bleeding as an unwanted side effect. In the opening case, Mr. Soprano developed a hematoma in his right thigh near the arterial access site at which eptifibatide was being infused. The expanding hematoma was caused by the excessive antiplatelet effect of a very high local concentration of eptifibatide at the infusion site. Importantly, the ability to reverse the effect of GPIIb–IIIa receptor inhibitors differs for the different agents. Because abciximab is an irreversible inhibitor of platelet function, and all the abciximab previously infused is already bound to platelets, infusion of fresh platelets after the drug has been stopped can reverse the antiplatelet effect. In contrast, because the two small molecule inhibitors (eptifibatide and tirofiban) bind the receptor reversibly and are infused in great stoichiometric excess of receptor number, infusion of fresh platelets simply offers new sites for the drug to bind. It is not practical to deliver a sufficient number of platelets to overwhelm the vast excess of drug present. Therefore, one must stop the drug infusion and wait for platelet function to return to normal as the drug is cleared. In the case of Mr. Soprano, no other measure could have been taken to reverse the effect of eptifibatide at the time his hematoma was recognized.

# ANTICOAGULANTS

As with antiplatelet agents, anticoagulants are used both to prevent and to treat thrombotic disease. There are four classes of anticoagulants in current clinical use—the immediate-acting, parenterally administered heparin class; the delayed-acting, orally administered warfarin class; hirudin and other direct inhibitors of thrombin; and activated protein C. Anticoagulants target various coagulation factors in the coagulation cascade, thereby interrupting the flow of the cascade and preventing the formation of a stable fibrin meshwork (secondary hemostatic plug). Because of the mechanisms of action of these drugs, bleeding is a side effect common to all anticoagulants.

## Immediate-Acting, Parenteral Anticoagulants: Unfractionated and Low Molecular Weight Heparins

### *Structure of Heparin*

**Heparin** is a sulfated mucopolysaccharide found in the secretory granules of mast cells. It is a highly sulfated polymer of alternating uronic acid and D-glucosamine. Heparin molecules are highly negatively charged; indeed, endogenous heparin is the strongest organic acid in the human body. Commercial preparations of heparin are quite heterogeneous, with molecular weights ranging from 1 to 30 kDa. Conventionally, commercially prepared heparins have been categorized into unfractionated (standard) heparin and low molecular weight (LMW) heparin. **Unfractionated heparin,** which is often prepared from bovine lung and porcine intestinal mucosa, ranges in molecular weight from 5 to 30 kDa. **LMW heparins** are prepared from standard heparin by gel filtration chromatography; their molecular weights range from 1 to 5 kDa.

### Mechanism of Action of Heparin

Heparin's mechanism of action depends on the presence of a specific plasma protease inhibitor, antithrombin III. Antithrombin III is actually a misnomer because, in addition to inactivating thrombin, antithrombin III inactivates other serine proteases including factors IXa, Xa, XIa, and XIIa. Antithrombin III can be considered as a stoichiometric "suicide trap" for these serine proteases. When one of the proteases encounters an antithrombin III molecule, the protease attacks a specific Arg–Ser peptide bond in the reactive site of the antithrombin and becomes "trapped" because of formation of a covalent ester bond between a serine residue of the protease and the arginine residue in the antithrombin III active site. This results in a stable 1:1 complex between the protease and antithrombin molecules, which prevents the protease from further participation in the coagulation cascade.

In the absence of heparin, the binding reaction between the protease and antithrombin III proceeds very slowly. Heparin, acting as a cofactor, accelerates the reaction by 1,000-fold. Heparin has two important physiologic functions: 1) it serves as a catalytic surface to which both antithrombin III and some serine proteases bind; and 2) it induces a conformational change in antithrombin III that makes the reactive site of this molecule more accessible to the attacking protease. The first step of the reaction involves the binding of the negatively charged heparin to a lysine-rich region (a region of positive charge) on antithrombin III. Thus, the interaction between heparin and antithrombin III is partly electrostatic. During the complexation reaction between the protease and the antithrombin, heparin is released from antithrombin III and is, therefore, able to catalyze additional protease–antithrombin III interactions. Thus, heparin is not consumed by the complexation reaction (Fig. 21-9).

Interestingly, heparins with different molecular weights have divergent anticoagulant activities. These divergent activities stem from the differential requirements for heparin binding exhibited by the inactivation of thrombin and factor Xa by antithrombin III (Fig. 21-14). To catalyze most efficiently the inactivation of thrombin by antithrombin III, a single molecule of heparin must bind simultaneously to both thrombin and antithrombin. This "scaffolding" function is required in addition to the heparin-induced conformational change in antithrombin III that renders this molecule susceptible to complexation with thrombin. In contrast, to catalyze the inactivation of factor Xa by antithrombin III, the heparin molecule must bind only to the antithrombin. The conformational change in antithrombin III induced by heparin binding is sufficient by itself to render the antithrombin susceptible to complexation with factor Xa. Thus, *LMW heparins,* which have an average molecular weight of 3–4 kDa and contain fewer than 18 monosaccharide units, *are capable of catalyzing efficiently the inactivation of factor Xa by antithrombin III, but are less efficient at catalyzing the inactivation of thrombin by*

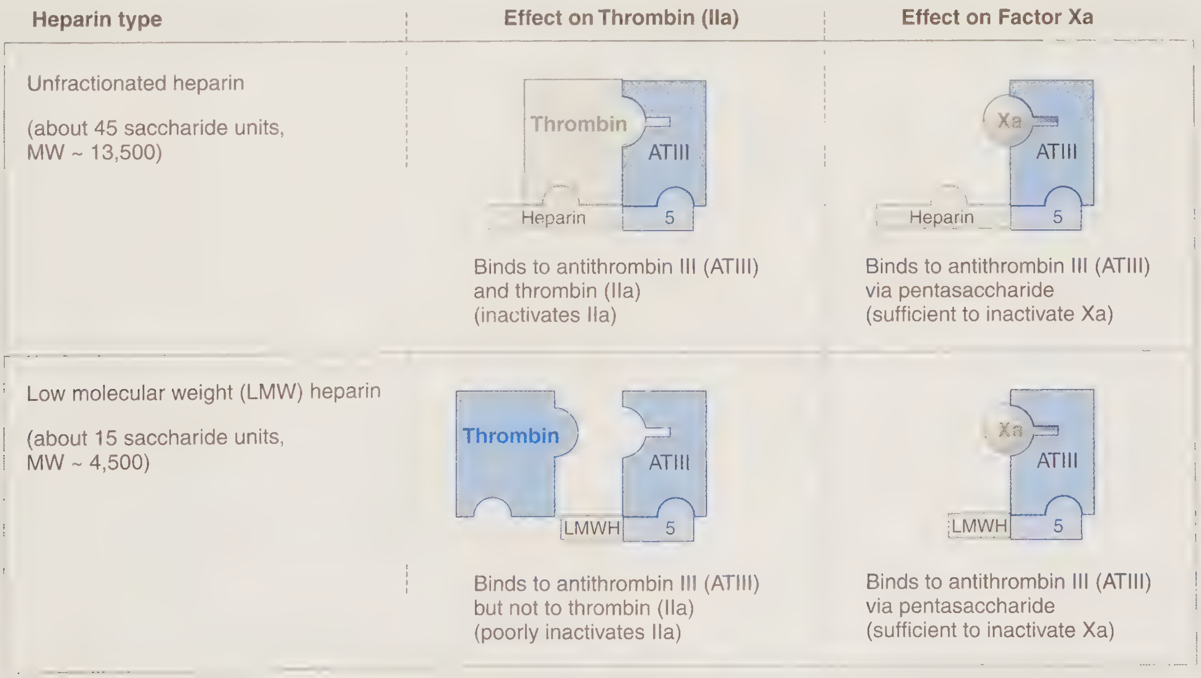

Heparin type
Effect on Thrombin (IIa)
Effect on Factor Xa
Unfractionated heparin
(about 45 saccharide units, MW ~ 13,500)
Thrombin
ATIII
Heparin
5
Xa
ATIII
Heparin
5
Binds to antithrombin III (ATIII) and thrombin (IIa) (inactivates IIa)
Binds to antithrombin III (ATIII) via pentasaccharide (sufficient to inactivate Xa)
Low molecular weight (LMW) heparin
(about 15 saccharide units, MW ~ 4,500)
Thrombin
ATIII
LMWH
5
Xa
ATIII
LMWH
5
Binds to antithrombin III (ATIII) but not to thrombin (IIa) (poorly inactivates IIa)
Binds to antithrombin III (ATIII) via pentasaccharide (sufficient to inactivate Xa)

Figure 21-14. Differential Effects of Unfractionated Heparin and Low Molecular Weight Heparin on Coagulation Factor Inactivation. **Effect on thrombin:** To catalyze the inactivation of thrombin, heparin must bind both to antithrombin III via a high-affinity pentasaccharide unit (5) and to thrombin via an additional 13 saccharide unit (here labeled "Heparin"). Low molecular weight heparin (LMWH) does not contain a sufficient number of saccharide units to bind thrombin, and, therefore, is a poor catalyst for thrombin inactivation. **Effect on factor Xa:** Inactivation of factor Xa requires only the binding of antithrombin III to the high-affinity pentasaccharide unit (5). Both unfractionated heparin and low molecular weight heparin are, therefore, able to catalyze the inactivation of factor Xa.

*antithrombin III.* In contrast, *unfractionated heparin,* which has an average molecular weight of 20 kDa and contains greater than 18 monosaccharide units, is of sufficient length to bind simultaneously to thrombin and antithrombin III, and *is therefore capable of catalyzing efficiently the inactivation of both thrombin and factor Xa by antithrombin III.* Quantitatively, LMW heparin has a threefold greater ratio of anti-Xa to anti-thrombin (IIa) activity than does unfractionated heparin. Both LMW heparin and unfractionated heparin use a pentasaccharide structure of high negative charge to bind antithrombin III and to induce the conformational change in antithrombin required for the complexation reactions. This pentasaccharide is currently being studied as an even more selective inactivator of factor Xa.

### Clinical Uses of Heparin

Heparin is used for both prophylaxis and treatment of thromboembolic diseases. For example, **enoxaparin,** a LMW heparin, is used to prevent deep venous thrombosis after hip replacement surgery, while both LMW and unfractionated heparin are used to prevent propagation of established thromboembolic disease such as deep venous thrombosis and pulmonary embolism. (An embolus is a thrombus that has broken off from its site of formation in the vasculature and has been carried through the bloodstream to a distant site, where it may lodge and cause significant obstruction of blood flow.) For prophylaxis against thrombosis, heparin is used at much lower doses than those indicated for the treatment of established thromboembolic disease. Because the enzymatic coagulation cascade functions as an amplification system (e.g., 1 unit of factor Xa generates 40 units of thrombin), the administration of relatively small amounts of circulating heparin at the first generation of factor Xa is highly effective. LMW heparin has a greater therapeutic index than does unfractionated heparin, especially when used for prophylaxis, because LMW heparin has a greater ratio of anti-Xa to antithrombin activity than does unfractionated heparin. Because heparin is highly negatively charged, neither unfractionated heparin nor LMW heparin can cross the epithelial cell layer of the gastrointestinal tract. Thus, heparin must be administered parenterally, usually via intravenous or subcutaneous routes. However, studies are currently underway to deliver LMW heparin orally using a carrier molecule to enhance bioavailability (see Chapter 50, Drug Delivery Mechanisms).

Heparin is often used in combination with antiplatelet agents in the treatment of acute coronary syndromes. For example, Mr. Soprano was treated with the antiplatelet agents aspirin and eptifibatide and with unfractionated heparin in an attempt to limit the extent of his myocardial infarction.

### Laboratory Monitoring of Heparin Therapy

Monitoring of unfractionated heparin therapy is important for maintaining the anticoagulant effect within the therapeutic range, because excessive heparin administration increases the risk of bleeding. Monitoring is usually performed by using the activated partial thromboplastin time (aPTT) assay. The aPTT is a simple test of the intrinsic and common pathways of coagulation. The patient's plasma is added to an excess of phospholipid, and fibrin forms at a normal rate only if the factors in the intrinsic and common pathways are present at normal levels. Increasing amounts of unfractionated heparin in the plasma prolong the time necessary for the formation of a fibrin clot. Somewhat surprisingly, the anticoagulant effect of LMW heparins is not accurately reflected by the aPTT assay. Accurate measurement of the anticoagulant effect of LMW heparins requires a specialized assay for antifactor Xa activity. In most clinical situations, however, it is not necessary to monitor the coagulation status of a patient treated with LMW heparins, because these agents have a greater therapeutic index than that of unfractionated heparin.

### Heparin Toxicity

Heparin is generally well tolerated by patients. As is true of other anticoagulants, the major adverse effect of heparin is bleeding. A small fraction of patients develop heparin-induced thrombocytopenia. In these cases, patients develop antibodies to platelets as a result of the heparin infusion, and the heparin-induced antiplatelet antibodies cause thrombocytopenia. In some cases, as described above, the heparin-induced antibodies can also cause platelet activation, endothelial injury, and a hypercoagulable state.

### Heparin Antagonist

**Protamine,** a low molecular weight polycationic protein, is a chemical antagonist of heparin. This agent forms a stable complex with the negatively charged heparin molecule through multiple electrostatic interactions. Protamine is administered intravenously to reverse rapidly the effects of heparin in situations of life-threatening hemorrhage or great heparin excess; for example, at the conclusion of coronary artery bypass graft surgery.

## Delayed-Acting, Oral Anticoagulants: Warfarin

In the early 1900s, farmers in Canada and the North Dakota plains adopted the practice of planting sweet clover instead of corn for fodder. In the winter months of 1921–1922, a fatal hemorrhagic disease was reported in cattle that foraged on sweet clover. The affected cattle could not form clots and died from fatal hemorrhage. In almost every case, it was found that the affected cattle had foraged on sweet clover that had been spoiled by the curing process. After an intensive investigation, scientist K. P. Link reported that the spoiled clover contained the natural anticoagulant 3,3′-methylene-bis-(4-hydroxycoumarin) or "dicumarol." Dicumarol and warfarin (a potent synthetic congener) were introduced during the 1940s as rodenticides and as oral anticoagulants. Because the oral anticoagulants act by affecting vitamin K-dependent reactions, it is important to understand how vitamin K functions.

### Mechanism of Action of Vitamin K

Vitamin K ("K" is derived from the German word "Koagulation") is required for the normal hepatic synthesis of four coagulation factors (II, VII, IX, and X), protein C, and protein S. The coagulation factors, protein C, and protein S are biologically inactive as unmodified polypeptides following protein synthesis on the ribosomes. These proteins gain biological activity after the post-translational carboxylation of their 9 to

12 amino-terminal glutamic acid residues. The gamma-carboxylated glutamate residues (but not the unmodified glutamate residues) are capable of binding $Ca^{2+}$ ions. $Ca^{2+}$ binding induces a conformational change in these proteins that is required for efficient binding of the proteins to phospholipid surfaces. The ability of the gamma-carboxylated molecules to bind $Ca^{2+}$ increases the enzymatic activity of coagulation factors IIa, VIIa, IXa, Xa, and protein Ca by approximately 1,000-fold. Thus, vitamin K-dependent carboxylation is crucial for the enzymatic activity of the four coagulation factors and protein C, and for the cofactor function of protein S.

The carboxylation reaction requires: (1) a precursor form of the target protein with its 9–12 amino-terminal glutamic acid residues; (2) carbon dioxide; (3) molecular oxygen; and (4) *reduced* vitamin K. The carboxylation reaction is schematically presented in Figure 21-15. During this reaction, vitamin K is oxidized to the inactive 2,3-epoxide. An enzyme, epoxide reductase, converts the inactive 2,3-epoxide into the active, reduced form of vitamin K. *Thus, the regeneration of reduced vitamin K is essential for the sustained synthesis of biologically functional clotting factors II, VII, IX, and X, all of which are critical components of the coagulation cascade.*

### Mechanism of Action of Oral Anticoagulants

Oral anticoagulants act on the carboxylation pathway, not by inhibiting the carboxylase directly, but by blocking the epoxide reductase that mediates the regeneration of reduced vitamin K

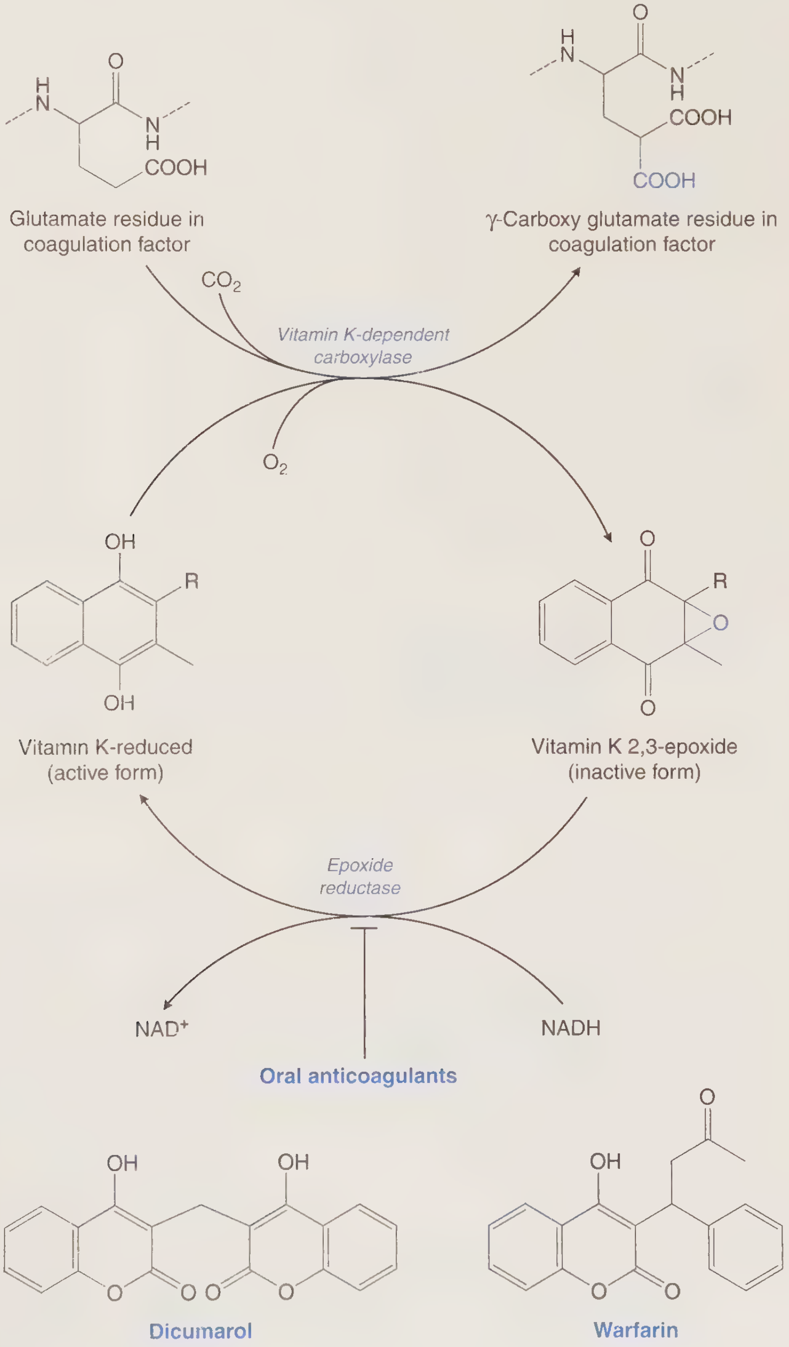

Glutamate residue in coagulation factor
γ-Carboxy glutamate residue in coagulation factor
COOH
COOH
$CO_2$
Vitamin K-dependent carboxylase
$O_2$
OH
OH
R
Vitamin K-reduced (active form)
O
O
R
Vitamin K 2,3-epoxide (inactive form)
Epoxide reductase
$NAD^+$
NADH
Oral anticoagulants
Dicumarol
Warfarin

**Figure 21-15. Mechanism of Action of Oral Anticoagulants.** Vitamin K is a necessary cofactor in the post-translational carboxylation of glutamate residues present on factors II, VII, IX, and X. During the carboxylation reaction, vitamin K is oxidized to the inactive 2,3-epoxide. The enzyme epoxide reductase converts the inactive vitamin K 2,3-epoxide into the active, reduced form of vitamin K. The regeneration of reduced vitamin K is essential for the sustained synthesis of biologically functional coagulation factors II, VII, IX, and X. Oral anticoagulants (warfarin and dicumarol) act on the carboxylation pathway by inhibiting the epoxide reductase that is required for the regeneration of reduced (active) vitamin K.

(Fig. 21-15). Because depletion of reduced vitamin K in the liver prevents the gamma–carboxylation reaction that is required for the synthesis of biologically active coagulation factors, the onset of action of the oral anticoagulants parallels the half-life of these coagulation factors in the circulation. Of the four affected clotting factors (II, VII, IX, and X), factor VII has the shortest half-life (6 hours). Thus, the pharmacologic effect of a single dose of warfarin is not manifested for approximately 18 to 24 hours (i.e., for 3 to 4 factor VII half-lives). This *delayed action* is one pharmacologic property that distinguishes the warfarin class of anticoagulants from the heparin class of anticoagulants.

Evidence from studies of long-term rodenticide use supports the hypothesis that the epoxide reductase is the molecular target of oral anticoagulant action. The use of oral anticoagulants as rodenticides has been a widespread practice in farming communities. In some areas of the United States, heavy rodenticide use has selected for a population of wild rodents that is resistant to 4-hydroxycoumarins. In vitro studies of tissues from these rodents have demonstrated a mutation in the rodent epoxide reductase that renders the enzyme resistant to inhibition by the anticoagulant. Similarly, a small population of patients is genetically resistant to oral anticoagulants because of mutations in their epoxide reductase gene. These patients require 10 to 20 times the usual dose of warfarin to achieve the desired anticoagulant effect.

### Warfarin

**Warfarin** is often administered to complete a course of anticoagulation that has been initiated with heparin and to prevent thrombosis in predisposed patients. Orally administered warfarin is nearly 100% bioavailable, and its levels in the blood peak at 0.5 to 4 hours after administration. *In the plasma, 99% of racemic warfarin is bound to plasma protein (albumin).* Warfarin has a relatively long elimination half-life (approximately 36 hours). The drug is hydroxylated by the cytochrome P450 system in the liver to inactive metabolites that are subsequently eliminated in the urine.

*Drug–drug interactions must be carefully considered in patients taking warfarin.* Because warfarin is highly albumin-bound in the plasma, co-administration of warfarin with other albumin-bound drugs can increase the free (unbound) plasma concentrations of both drugs. In addition, because warfarin is metabolized by P450 enzymes in the liver, co-administration of warfarin with drugs that induce and/or compete for P450 metabolism can affect the plasma concentrations of both drugs. Table 21-2 lists some of the major interactions between warfarin and other drugs.

Among the adverse effects of warfarin, bleeding is the most serious and predictable toxicity. Withdrawal of the drug may be recommended for patients who suffer from repeated bleeding episodes at therapeutic drug concentrations. For severe hemorrhage, patients should promptly receive fresh frozen plasma, which contains biologically functional clotting factors II, VII, IX, and X. Warfarin should *never* be given to pregnant women because it can cross the placenta and cause a hemorrhagic disorder in the fetus. In addition, newborns who were exposed to warfarin in utero may have serious congenital defects characterized by abnormal bone formation (certain bone matrix proteins are γ-carboxylated). Last, warfarin may rarely cause skin necrosis as a result of widespread thrombosis in the microvasculature. The fact that warfarin can cause thrombosis may seem paradoxical. Recall that, in addition to inhibiting the synthesis of biologically active coagulation factors II, VII, IX, and X, warfarin also prevents the synthesis of biologically active proteins C and S, which are natural anticoagulants. In patients who are genetically deficient in protein C or protein S (e.g., heterozygotes for protein C deficiency), an imbalance between warfarin's effects on coagulation fac-

TABLE 21-2A Examples of Drugs That Diminish Warfarin's Anticoagulant Effect

| DRUG OR DRUG CLASS | MECHANISM |
|---|---|
| Cholestyramine | Inhibits warfarin absorption in the GI tract |
| Barbiturates | Accelerates warfarin metabolism by inducing hepatic P450 enzymes |
| Vitamin K (reduced) | Bypasses warfarin's inhibition of epoxide reductase |

TABLE 21-2B Examples of Drugs That Enhance Warfarin's Anticoagulant Effect

| DRUG OR DRUG CLASS | MECHANISM |
|---|---|
| Chloral hydral | Displaces warfarin from plasma albumin |
| Chloramphenicol | Decreases warfarin metabolism by inhibiting hepatic P450 enzymes |
| Broad-spectrum antibiotics | Reduce availability of vitamin K in the GI tract |
| Anabolic steroids (testosterone) | Inhibit synthesis and increase degradation of clotting factors |

tors and its effects on proteins C and S may lead to microvascular thrombosis and subsequent skin necrosis.

Because warfarin has a narrow therapeutic index and participates in numerous drug–drug interactions, the pharmacodynamic (functional) effect of chronic warfarin therapy must be monitored regularly (on the order of every 2–4 weeks). Monitoring is most easily performed by using the prothrombin time (PT), expressed as the international normalized ratio (INR) of the prothrombin time in the patient sample to that in a control sample. The PT is a simple test of the extrinsic and common pathways of coagulation. The patient's plasma is added to a crude preparation of tissue factor, and the time for formation of a fibrin clot is measured. Warfarin prolongs the PT mainly because it decreases the amount of biologically functional factor VII in the plasma. (Recall that factor VII is the vitamin K-dependent coagulation factor with the shortest half-life.)

### Direct Thrombin Inhibitor: Hirudin

As noted above, thrombin plays a number of critical roles in the hemostatic process (Fig. 21-8). This clotting factor: (1) proteolytically converts fibrinogen to fibrin; (2) activates factor XIII, which cross-links fibrin polymers to form a stable clot; (3) activates platelets; and (4) induces endothelial release of $PGI_2$, t-PA, and PAI-1. Thus, direct thrombin inhibitors would be expected to have profound effects on coagulation.

**Hirudin,** a direct thrombin inhibitor, is a recombinant 65-amino acid polypeptide derived from the medicinal leech. For years, surgeons have used medicinal leeches to prevent thrombosis in the fine vessels of reattached digits. Hirudin **(lepirudin)** binds with high affinity to two sites on the thrombin molecule—the enzymatic active site and the "exosite." Hirudin binding to thrombin prevents the thrombin-mediated activation of fibrinogen and factor XIII. Hirudin is a highly effective anticoagulant because it can inhibit both free and *fibrin-bound* thrombin in developing clots.

As could be predicted from its mechanism of action, bleeding is the major side effect of hirudin. Currently, the FDA has approved the use of hirudin only in patients who cannot tolerate heparin, for example, patients in whom heparin-induced thrombocytopenia has been demonstrated. Other recently approved direct thrombin inhibitors include **argatroban** and the synthetic hirudin analogue **bivalirudin.**

### Recombinant Activated Protein C (r-APC)

As described above, endogenously activated protein C exerts an anticoagulant effect by inhibiting factors Va and VIIIa. Activated protein C (APC) has also been shown to reduce the amount of circulating plasminogen activator inhibitor type 1, thereby enhancing fibrinolysis. Finally, APC has been found to reduce inflammation by inhibiting the release of tumor necrosis factor α from monocytes. Because enhanced coagulability and inflammation are both hallmarks of septic shock, APC has been tested both in animal models of this disorder and in humans. **Recombinant activated protein C (r-APC)** has been found to significantly reduce mortality in patients at high risk of death from septic shock, and the FDA has recently approved APC for the treatment of patients with severe sepsis who demonstrate evidence of acute organ dysfunction, shock, oliguria, acidosis, and hypoxemia. As is the case with other anticoagulants, r-APC increases the risk of bleeding. This agent is, therefore, contraindicated in patients who have recently undergone a surgical procedure and in those with chronic liver failure, kidney failure, or thrombocytopenia.

## THROMBOLYTIC AGENTS

Although the heparin and warfarin classes of anticoagulants are effective in preventing the formation and propagation of thrombi, these agents are generally inactive against preexisting clots. Thrombolytic agents are used to lyse already-formed clots and, thereby, to restore the patency of an obstructed vessel before distal tissue necrosis occurs. Thrombolytic agents act by activating plasminogen proteolytically to form plasmin (Fig. 21-10). As noted above, plasmin is a relatively nonspecific protease that digests fibrin to fibrin degradation products. Unfortunately, thrombolytic therapy has the potential to dissolve not only pathologic thrombi, but also physiologically appropriate fibrin clots that have formed in response to vascular injury. Thus, the use of thrombolytic agents can lead to hemorrhage of varying severity.

### Streptokinase

**Streptokinase** is a protein produced by β-hemolytic streptococci as a component of that organism's tissue-destroying machinery. The pharmacologic action of streptokinase involves two steps—complexation and cleavage. In the complexation reaction, streptokinase forms a stable, noncovalent 1:1 complex with plasminogen. The complexation reaction produces a conformational change in plasminogen that exposes this protein's proteolytically active site. Streptokinase-complexed plasminogen, with its active site exposed and available, can then proteolytically cleave *other* plasminogen molecules to plasmin. In fact, the thermodynamically stable streptokinase: plasminogen complex is the most catalytically efficient plasminogen activator in vitro.

Although streptokinase exerts its most dramatic and potentially beneficial effects in fresh thrombi, its use has been limited by two factors. First, streptokinase is a foreign protein that is capable of eliciting antigenic responses in humans upon repeated administration. Second, its thrombolytic actions are relatively nonspecific and can result in systemic fibrinolysis.

### Urokinase

**Urokinase** is an endogenous protease obtained from human fetal kidney cell cultures. It is also found in human urine. This agent acts by converting plasminogen to plasmin directly. Current interest in exploring the use of urokinase as a therapeutic agent is limited because this agent causes systemic fibrinolysis by virtue of its lack of fibrin specificity.

### Recombinant Tissue Plasminogen Activator (t-PA)

An ideal thrombolytic agent would be nonantigenic and would cause local fibrinolysis only at the site of a pathologic thrombus. Tissue plasminogen activator (t-PA) approximates these goals. t-PA is a serine protease produced by human endothelial cells; thus, t-PA is not antigenic. t-PA binds to newly formed (fresh) thrombi with high affinity, causing fibrinolysis at the site of a thrombus. Once bound to the fresh thrombus, t-PA undergoes a conformational change that renders it a potent activator of plasminogen. In contrast, t-PA is a poor activator of plasminogen in the absence of fibrin-binding.

Recombinant DNA technology has allowed the production of **recombinant t-PA.** Recombinant t-PA is effective in recanalizing occluded coronary arteries, limiting cardiac dysfunction, and reducing mortality following an acute myocardial infarction. At pharmacologic doses, however, recombinant t-PA can generate a systemic lytic state and (as with other thrombolytic agents) cause unwanted bleeding, including cerebral hemorrhage. Thus, its use is contraindicated in patients who have had a recent stroke.

### Anisoylated Plasminogen Streptokinase Activator Complex (APSAC)

**APSAC** is a complex of streptokinase and plasminogen that is acetylated at the active site of plasminogen. This acetylation prevents the proteolytic activity of plasminogen. APSAC is administered intravenously and circulates in the bloodstream as an inactive complex (prodrug). The circulating complex binds to fibrin with high affinity and specificity via the fibrin binding site on the plasminogen component of the complex. The complex then undergoes spontaneous, nonenzymatic hydrolysis, in which the plasminogen active site is de-acetylated with a half-time of 90 minutes. De-acetylated APSAC is thereby activated at the site of the fibrin clot.

## *Conclusion and Future Directions*

Hemostasis is a highly regulated process that maintains the fluidity of blood in normal vessels and initiates rapid formation of a stable fibrin-based clot in response to vascular injury. Pathologic thrombosis results from endothelial injury, abnormal blood flow, and hypercoagulability. Antiplatelet agents, anticoagulants, and thrombolytic agents target different stages of thrombosis and thrombolysis. Antiplatelet agents interfere with platelet adhesion, the platelet release reaction, and platelet aggregation; these agents can provide powerful prophylaxis against thrombosis in susceptible individuals. Anticoagulants primarily target plasma coagulation factors and disrupt the coagulation cascade by inhibiting crucial intermediates. After a fibrin clot has been established, thrombolytic agents mediate the dissolution of the clot by promoting the conversion of plasminogen to plasmin. These classes of pharmacologic agents can be administered either individually or in combination, to prevent or disrupt thrombosis and to restore the patency of blood vessels occluded by thrombus.

Future development of new antiplatelet, anticoagulant, and thrombolytic agents will be forced to contend with two major constraints. First, for many clinical indications in this field, highly effective, orally available, and inexpensive therapeutic agents are already available; these include the antiplatelet drug aspirin and the anticoagulant warfarin. Second, virtually every antithrombotic and thrombolytic agent is associated with the mechanism-based toxicity of bleeding, and this side effect is very likely to plague new agents under development. Nonetheless, opportunities remain for the development of safer and more effective therapies. It is likely that pharmacogenomic techniques (see Chapter 52) will be capable of identifying individuals in the population who carry an elevated genetic risk of thrombosis, and such individuals may benefit from long-term antithrombotic treatment. Combinations of antiplatelet agents, low molecular weight heparins, and new agents that target currently unexploited components of hemostasis (such as tissue factor pathway inhibitor) could all be useful in these settings. At the other end of the spectrum, there remains a great need for new agents that can achieve rapid, noninvasive, convenient, and selective lysis of acute thromboses associated with such life-threatening emergencies as acute myocardial infarction and stroke. Carefully designed clinical trials will be critical to optimize the indications, dose, and duration of treatment for such drugs and drug combinations.

## *Suggested Readings*

Brass L. The molecular basis of platelet activation. In: Hoffman R et al., eds. Hematology: Basic Principles and Practice. 3rd Ed. Philadelphia: Churchill Livingstone, 2000:1753–1770. (*Detailed and mechanistic description of platelet activation*)

Hirsh J, Lee AYY. How we diagnose and treat deep vein thrombosis. Blood 2002;99:3102–3110. (Current treatment options for deep vein thrombosis)

Mitchell RN, Cotran RS. Hemodynamic disorders, thrombosis, and shock. In: Cotran R et al., eds. Robbins Pathologic Basis of Disease. 6th Ed. Philadelphia: W.B. Saunders Company, 1999:113–138. (*Useful overview of various hemodynamic disorders*)

Rosenberg R, Aird W. Vascular-bed–specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555–1564. (*Thorough review of the pathophysiology of hypercoagulable states*)

Weitz J. Low molecular weight heparins. N Engl J Med 1997;337: 688–698. (*Comprehensive review of the mechanism of action, use, and toxicity of LMW heparins*)

Weitz JI, Crowther M. Direct thrombin inhibitors. Thrombosis Research 2002;106:V275–V284. (Summarizes clinical trial data on direct thrombin inhibitors)

## Drug Summary Table Pharmacology of Hemostasis and Thrombosis

| Drug | Clinical Use | Side Effects/Toxicity | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ANTIPLATELET AGENTS**<br>*Mechanism—Inhibit platelet aggregation; see Notes for individual drug mechanisms* | | | | |
| **Aspirin** | Prevent clot formation | Bleeding (especially GI bleeding), nausea | | Irreversibly inhibits platelet thromboxane $A_2$ synthesis by acetylating cyclooxygenase |
| **Cilostazol**<br>**Dipyridamole** | Prevent thrombus formation on prosthetic heart valves | Dizziness, angina, abdominal pain | | Increase platelet cAMP by inhibiting phosphodiesterase |
| **Ticlopidine**<br>**Clopidogrel** | Acute cerebral ischemia (ticlopidine)<br>Atherosclerotic vascular disease (clopidogrel) | GI irritation, bleeding<br>Neutropenia, agranulocytosis (ticlopidine) | | Covalently modify the platelet ADP receptor and inhibit ADP-induced binding of fibrinogen to platelet membrane |
| **Eptifibatide**<br>**Abciximab**<br>**Tirofiban** | PTCA (see Notes) | Bleeding | | Antagonize GPIIb–IIIa receptor and prevent fibrinogen binding<br>Used mainly in patients undergoing percutaneous transluminal angioplasty (PTCA) |
| **ANTICOAGULANTS**<br>*Mechanism—Inhibit steps in the coagulation cascade; see Notes for individual drug mechanisms* | | | | |
| **Unfractionated heparin** | Immediate anticoagulation—treatment of pulmonary embolism and deep venous thrombosis | Bleeding, hypersensitivity, heparin-induced thrombocytopenia | | Binds to antithrombin III and accelerates inactivation of IIa, IXa, Xa, XIa, and XIIa<br>aPTT is used to monitor the action of heparin<br>Protamine sulfate counteracts the effects of heparin<br>Preferred anticoagulant during pregnancy |
| **Low molecular weight heparin (LMWH)** | Prophylaxis against deep venous thrombosis | Bleeding, lower incidence of heparin-induced thrombocytopenia | | Binds to antithrombin III and accelerates preferential inactivation of Xa<br>aPTT is not a useful measure of LMWH action |
| **Warfarin** | Long-term anticoagulation | Hemorrhage | Contraindicated in pregnant women (crosses placenta) | Interferes with synthesis of vitamin K, which is needed for activation of factors II, VII, IX, and X, and proteins C and S<br>INR (international normalized ratio) is used to monitor effects of warfarin<br>Vitamin K or fresh frozen plasma reverses effects of warfarin |
| **Hirudin** | Patients who cannot tolerate heparin | Hemorrhage | | Binds directly to and inhibits free and fibrin-bound thrombin |
| **Recombinant human activated protein C (r-APC)** | Severe sepsis | Hemorrhage | | Inhibits factors Va and VIIIa and reduces the amount of plasminogen activator inhibitor type 1<br>Has some anti-inflammatory effects through inhibiting monocyte release of TNF-α |

*(Continued)*

**Drug Summary Table** **Pharmacology of Hemostasis and Thrombosis** *(Continued)*

| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
|---|---|---|---|---|
| **THROMBOLYTIC AGENTS**<br>*Mechanism—Convert plasminogen to plasmin* | | | | |
| **Streptokinase** | Acute pulmonary embolism, deep venous thrombosis, acute myocardial infarction (MI) | Systemic bleeding | | Streptokinase-plasminogen complex converts uncomplexed plasminogen into plasmin<br>Derived from group C β-hemolytic streptococci; can be immunogenic<br>Aminocaproic acid reverses the action of streptokinase |
| **Urokinase** | Pulmonary embolism and deep venous thrombosis | Systemic fibrinolysis | | Directly converts plasminogen to plasmin<br>Use of urokinase is limited because of lack of fibrin specificity<br>Aminocaproic acid reverses the action of urokinase |
| **Recombinant tissue plasminogen activator (t-PA)** | Acute MI | Bleeding | | Binds to and activates fibrin-bound plasminogen and not free plasminogen<br>Human sequence<br>Aminocaproic acid reverses the action of t-PA |
| **Anisoylated plasminogen streptokinase activator complex (APSAC)** | Acute MI | Bleeding | | The complex binds to fibrin and then undergoes a spontaneous hydrolysis where the plasminogen active site is deacetylated and the plasminogen is activated at the fibrin site<br>Aminocaproic acid reverses the action of APSAC |

# 22

# Pharmacology of Cholesterol and Lipoprotein Metabolism

Ehrin J. Armstrong, Patrick K. Safo and Frank M. Sacks

**Introduction**
**Case**
**Biochemistry and Physiology of Cholesterol and Lipoprotein Metabolism**
  - Dietary Cholesterol and Fatty Acid Absorption
  - Endogenous Lipoprotein Production
  - Role of HDL
**Pathophysiology**
  - Hypercholesterolemia
  - Hypertriglyceridemia
  - Mixed Hyperlipidemia
  - Disorders of HDL Metabolism
  - Secondary Hyperlipidemia
**Pharmacologic Classes and Agents**
  - Inhibitors of Bile Acid Absorption
  - Inhibitors of Cholesterol Absorption
  - Inhibitors of Cholesterol Synthesis
  - Fibrates
  - Niacin
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Lipids are essential for membrane synthesis, maintenance of membrane integrity, as an energy source, as hormone precursors, and as signaling molecules. To aid transport through the relatively aqueous blood, lipids are transported as cholesteryl esters or triglycerides within lipoproteins. However, increased levels of lipoproteins in the circulation are associated strongly with atherosclerosis. Much of the prevalence of cardiovascular disease (CVD), the leading cause of death in the US and most Western countries, can be attributed to elevated levels of LDL and triglycerides, and to decreased levels of HDL. The major portion of this increased risk may be due to Western diets and a sedentary lifestyle; while several genetic causes of hyperlipidemia have been identified, the role of genetics in the common forms of hyperlipidemia is still being elucidated. However, genetics clearly modifies the sensitivity of individuals to adverse diet habits and lifestyle. This chapter highlights the biochemistry and physiology of cholesterol and lipoproteins, with an emphasis on the role of lipoproteins in atherogenesis, and the pharmacologic interventions that can ameliorate hyperlipidemia and, ultimately, lower the incidence of mortality from cardiovascular disease. The role of lipids as signaling molecules is discussed in Chapter 40, Pharmacology of Eicosanoids.

## Case

In June 1998 Jake, a 29-year-old construction worker, makes an appointment to see Dr. Cush. Jake complains of hard, elevated swellings around his Achilles tendon that seem to rub constantly against his construction boots. Jake had been hesitant to see the doctor (his last appointment was 10 years ago), but he remembers that his dad, who died at age 42 of a heart attack, had similar swellings. On examination, Dr. Cush recognizes the Achilles swellings as xanthomas (lipid deposits); the physical exam is otherwise within normal limits. Jake comments that his daily diet is quite "fatty," including 3–4 donuts per day and frequent hamburgers. Dr. Cush explains that the xanthomas on Jake's feet are the result of cholesterol ester deposition, probably from high cholesterol levels in his blood. Dr. Cush orders a fasting plasma cholesterol level, and recommends that Jake reduce his intake of foods high in saturated fat and cholesterol, and increase his intake of poultry, fish, whole cereal grains, fruits, and vegetables. Jake has gained about 15 pounds since he was 19, and has a small "paunch." Dr. Cush recommends regular exercise and weight loss.

Results of the blood test show total plasma cholesterol of 300 mg/dL (normal, <200), with elevated LDL of

250 mg/dL (desirable, <130), low HDL (35 mg/dL), and normal levels of triglycerides and VLDL. Based on these test results, the Achilles heel xanthomas, and a positive family history for an early myocardial infarction, Dr. Cush tells Jake that he likely has an inherited disorder of cholesterol metabolism, probably familial hypercholesterolemia. This disease puts Jake at high risk for early atherosclerosis and myocardial infarction, but aggressive lowering of cholesterol levels can abrogate many of the disease sequelae. The low HDL also contributes to his increased risk of cardiovascular disease. In addition to the dietary changes, Dr. Cush prescribes a statin to help reduce Jake's cholesterol. A starting dose of a statin reduces his LDL by 30% to 175 mg/dL, while HDL slightly increases. Dr. Cush then increases the statin dose, and this produces an additional 12% reduction in LDL. Because LDL has still not reached <130 mg/dL, and HDL remains low, Dr. Cush adds extended-release niacin. After these modifications, Jake's LDL drops below 130 and his HDL increases to 45 mg/dL, a reasonable goal. Jake experiences cutaneous flushing during the first few months of niacin treatment, but after that period, he has only occasional episodes.

## QUESTIONS

- **1.** What is the etiology of familial hypercholesterolemia?
- **2.** How do high cholesterol levels predispose to cardiovascular disease?
- **3.** How do statins and niacin act pharmacologically?
- **4.** What side effects of concomitant statin and niacin therapy should Jake be concerned about?

## BIOCHEMISTRY AND PHYSIOLOGY OF CHOLESTEROL AND LIPOPROTEIN METABOLISM

Lipoproteins are carrier molecules used for transporting triglycerides and cholesterol through the blood. Circulating lipoproteins can be differentiated on the basis of density, size, and protein content (Table 22-1). As a trend, larger, less dense lipoproteins have a greater percentage composition of triglyceride; chylomicrons consist of 85% triglyceride and are the largest and least dense lipoprotein subclass, while high density lipoproteins (HDL) possess only 5% triglyceride and are the smallest, highest-density class of lipoproteins.

Structurally, lipoproteins are microscopic spherical particles ranging from 7 to 100 nm in diameter. Each lipoprotein particle consists of a lipid membrane, a hydrophobic core, and certain apolipoproteins (Fig. 22-1). The lipid membrane contains polar lipids, such as free cholesterol and phospholipids, arranged in a monolayer. The hydrophobic core of a lipoprotein contains the cholesteryl esters (cholesterol molecules linked by an ester bond to a fatty acid) and triglycerides (a glycerol molecule linked to three fatty acids). The

**TABLE 22-1** Lipoprotein Classes

| TYPE | ORIGIN | MAJOR LIPIDS | MAJOR APOLIPOPROTEINS | CATABOLISM |
|---|---|---|---|---|
| Chylomicrons | Intestine | 85% Triglyceride | B48, AI, AIV, E, CI, CII, CIII | Hydrolysis of triglyceride (lipoprotein lipase) |
| Chylomicron remnants | From chylomicrons | 40–60% Triglyceride<br>20% Cholesterol | B48, AI, AIV, E, CI, CII, CIII | Uptake by liver (apoE via LDL receptor) |
| VLDL | Liver | 55% Triglyceride<br>20% Cholesterol | B100, E, CI, CII, CIII | Hydrolysis of triglyceride (lipoprotein lipase) to IDL; direct uptake by liver (apoE and B100 via LDL receptor) |
| IDL | From VLDL and liver | 35% Cholesterol<br>25% Triglyceride | B100, E, CI, CII, CIII | → LDL (hepatic lipase)<br>→ Liver (apoE and B100) |
| LDL | From IDL and liver | 60% Cholesterol<br>5% Triglyceride | B100, E, CI, CII, CIII | Uptake: liver (apoB100 via LDL receptor) |
| HDL | Liver, intestine, and plasma | 35% Phospholipid<br>20% Cholesterol<br>5% Triglyceride | AI, AII, CI, CII, CIII, E | 1. Uptake of cholesterol ester by hepatocytes (SR-B1)<br>2. Transfer of cholesteryl ester (CETP) to LDL, IDL, and VLDL<br>3. Lipolysis of TG and CE by hepatic lipase and uptake by liver<br>4. Clearance of HDL particle by liver and kidney |

Each lipoprotein class has a characteristic lipid composition and set of apolipoproteins. ApoB lipoproteins are in chylomicrons, VLDL, IDL, and LDL; whereas, apoA lipoproteins are in HDL, as their major and essential apolipoprotein component. The other associated apolipoproteins modulate the metabolism. For example, the presence of apoCI or CIII on some chylomicrons, VLDL, IDL, and LDL slows metabolism by lipoprotein lipase and LDL receptors; whereas, apoE accelerates uptake of these lipoproteins by the liver. CETP on HDL is crucial for cholesterol transfer. Some of the most crucial enzymes for lipoprotein catabolism are listed in parentheses. (VLDL: very low-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CETP: cholesterol ester transfer protein; TG: triglyceride; CE: cholesteryl ester.)

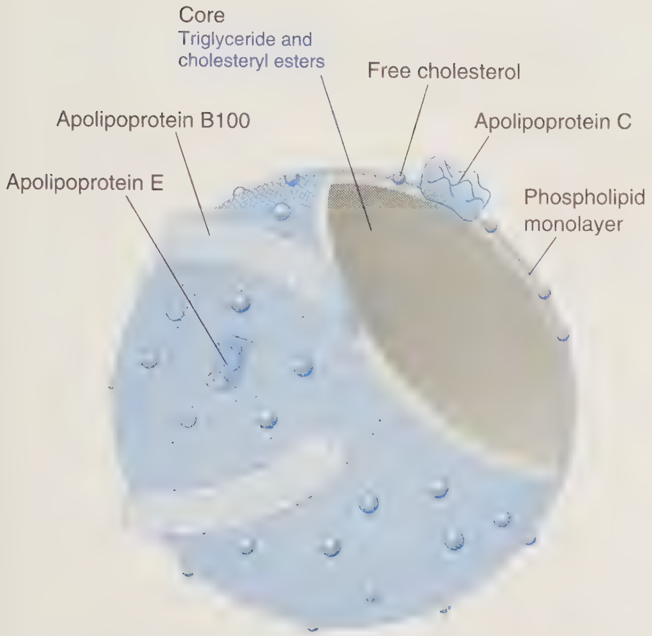


**Figure 22-1. Structure of Lipoprotein Particles.** Lipoproteins are spherical particles (7–100 nm in diameter) that transport such hydrophobic molecules as cholesterol, triglycerides, and fat-soluble vitamins. Each lipoprotein particle subclass has a hydrophilic monolayer surface, composed of phospholipid and cholesterol, that shields a hydrophobic core of triglyceride and cholesteryl esters. Lipoproteins contain apolipoproteins (also called apoproteins) that associate with the surface lipids and hydrophobic core through multiple amphipathic membrane-spanning segments. These apolipoproteins provide structural stability to the lipoprotein particle and act as ligands for specific cell surface receptors or as cofactors for enzymatic reactions. In the example shown, a very low-density lipoprotein particle (VLDL) contains apolipoprotein E, apolipoprotein B100, and apolipoprotein C (CI, CII, and CIII).

**apolipoproteins** (also referred to as apoproteins) are protein molecules that integrate into the lipid membrane of lipoproteins. These apolipoproteins act as receptor ligands for lipoprotein particles; the characteristic set of apolipoproteins associated with a given lipoprotein class largely determines the functions of the lipoprotein. For example, all low-density lipoproteins (LDL) contain an apoB100 molecule; this apolipoprotein is a ligand for the low-density lipoprotein receptor (LDL-R, discussed below), thus allowing cellular cholesterol uptake. The major apolipoproteins and their functions are listed in Table 22-2.

Several groups of lipoproteins are listed in Table 22-1. From a metabolic perspective, these particles can be divided into lipoproteins that participate in dietary lipid absorption and delivery to muscle and fat tissue (chylomicrons and chylomicron remnants) and lipoproteins that are involved in lipid production and transport (VLDL, IDL, and LDL). HDL serves as a link between these metabolic pathways by transferring apolipoproteins and cholesterol among the different lipoprotein subclasses and to the liver. The following discussion presents each lipoprotein class in the context of dietary lipid metabolism. The molecular interactions between apolipoproteins and their receptors are emphasized as the basis for understanding cholesterol and triglyceride metabolism.

## DIETARY CHOLESTEROL AND FATTY ACID ABSORPTION

During digestion, cholesteryl esters and triglycerides in food are hydrolyzed to cholesterol and free fatty acids. Bile salts and phospholipids (bile components) secreted into the small intestine solubilize these lipids and facilitate absorption into enterocytes of the duodenum and jejunum. Long-chain fatty acids then undergo packaging in the enterocyte; medium-chain fatty acids are absorbed directly into the portal blood, where they are metabolized by the liver. The packaging process consists of re-esterifying free long-chain fatty acids into water-insoluble triglycerides; the absorbed dietary cholesterol is also esterified to cholesteryl ester. These esterified lipids are then packaged together in the gut epithelial cell Golgi apparatus with the apolipoproteins **apoB48,** the required structural protein of chylomicrons, and **apoAI.**

The apoB48, apoAI, triglyceride, and cholesteryl ester complex is then secreted by enterocytes as a **chylomicron** (Fig. 22-2). Chylomicrons are secreted into lymphatics and carried by the thoracic duct to the venous circulation. In the circulation, apoE, apoCI, apoCII, and apoCIII are transferred from HDL (discussed below) onto chylomicrons. **Lipoprotein lipase (LPL),** an enzyme activated by **apoCII** and located on the capillary endothelial surface in adipose and muscle tissues, hydrolyzes triglycerides from circulating chylomicrons. This liberates free fatty acids and glycerol, which are then taken up by neighboring cells for use in intermediary metabolism to generate ATP, and for energy storage.

As LPL continues to hydrolyze triglycerides from chylomicrons, the chylomicrons progressively possess a smaller percentage of triglyceride and a relatively larger percentage of cholesterol. As chylomicrons become relatively triglyceride poor, the apolioproteins apoAI, apoCI, apoCII, and apoCIII are transferred to HDL. Eventually, the chylomicron is termed a chylomicron remnant. These remnants are taken up by the liver in an interaction mediated by the binding of apoE to hepatic LDL-R or LDL-R related protein (an apoE receptor), and the chylomicron remnant is removed from the circulation.

## ENDOGENOUS LIPOPROTEIN PRODUCTION

The level of chylomicrons in the bloodstream is largely dependent on the amount of dietary fat intake; this underscores the essential role that chylomicrons play in absorption of dietary fat. In comparison, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) are synthesized de novo by the liver as a means of transporting fatty acids and cholesterol to peripheral tissues. For this reason, the metabolism of these lipoproteins is often referred to as the endogenous lipid production pathway. (The distinction is, however, somewhat arbitrary because increased dietary fat intake will lead to greater fatty acid flux to the liver, and, in turn, increase synthesis of endogenous pathway lipoproteins.)

Hepatocytes initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after a fatty meal or in situations of increased lipolysis). Interestingly, dietary carbohydrate also stimulates this process. Cholesterol and fatty acid in the hepatocyte fuse with

TABLE 22-2 Major Apolipoproteins

| APOLIPOPROTEIN | SITE OF SYNTHESIS | LIPOPROTEINS | FUNCTION |
|---|---|---|---|
| B48 | Intestine | Chylomicrons and remnants | Structural protein for chylomicrons |
| B100 | Liver | VLDL, IDL, LDL | Ligand for LDL receptor (LDL-R) |
| E | Liver<br>Brain | Chylomicrons and remnants, VLDL, IDL, some HDL | Ligand for LDL-R and remnant receptor (LRP) |
| CI | Liver | Chylomicrons<br>VLDL, IDL, LDL, HDL | Activates LCAT<br>Modulates apoE activity<br>Blocks uptake by LDL receptors. Small amounts on IDL and LDL. Function on HDL is unknown. |
| CII | Liver | Chylomicrons<br>VLDL, IDL, LDL, HDL | Required cofactor for lipoprotein lipase (LPL). Blocks uptake by LDL receptors. Small amounts on IDL and LDL. Function on HDL unknown. |
| CIII | Liver | Chylomicrons<br>VLDL, IDL, LDL, HDL | Inhibits LPL. Blocks uptake by LDL receptors. Small amounts on IDL and LDL. Function on HDL unknown. |
| AI | Liver<br>Intestine | Chylomicrons<br>HDL | Activates LCAT. Activates reverse cholesterol transport by SR-B1. Required for HDL structure.<br>Inhibits apoE-receptor association |
| AII | Liver | HDL; small amounts on VLDL | May inhibit plasminogen → plasmin. Blocks catabolism by LDL receptors |
| apo(a) | Liver | Lp(a) | |

Apolipoproteins confer functional specificity to lipoprotein particles by acting as ligands for receptors, as cofactors for enzymes, or as structural proteins. (VLDL: very low-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; LRP: LDL receptor-related protein; LCAT: lecithin:cholesterol acyltransferase.)

apolipoprotein B100 polypeptides, in a co-translational reaction that is dependent on the enzyme **microsomal triglyceride transfer protein** (MTP), to form a nascent VLDL particle. This nascent VLDL particle coalesces with larger triglyceride droplets and is subsequently secreted into the circulation (Fig. 22-3). VLDL particles also possess the lipoproteins apoE, apoCI, apoCII, and apoCIII; the majority of these apolipoproteins attach to VLDL in the hepatocyte before secretion, but some may be acquired from HDL during circulation through the bloodstream. IDL and LDL are also secreted by the liver, although most IDL and LDL in the bloodstream are derived from VLDL.

As VLDL circulates, LPL expressed in endothelial cell membranes of adipose and muscle tissue hydrolyzes the triglyceride core of VLDL into free fatty acids (Fig. 22-3). The apolipoproteins apoAII, apoCI, apoCII, and apoCIII are also transferred to HDL. In a manner analogous to chylomicron metabolism, VLDL particles become smaller and relatively triglyceride poor, resulting in the formation of VLDL remnants, IDL, and LDL. IDL can either be internalized by hepatocytes or converted to LDL. Internalization of IDL is dependent on hepatic LDL-R, with apoB100 or apoE serving as a ligand. Conversion to LDL occurs as hepatic lipase hydrolyzes the triglyceride core of IDL. *Thus, most circulating LDL is derived from VLDL.* LDL particles are characterized by a significant (60%) cholesterol content and the presence of apoB100. A small percentage of LDL contains one or more of the apoCs; these particles are large and likely to be partially lipolyzed remnants of VLDL. Because of its long residence time in the circulation (2–4 days), LDL accounts for approximately 65%–75% of total plasma cholesterol.

LDL can be removed from the circulation by either the LDL receptor or the macrophage scavenger receptor (Fig. 22-3). The LDL receptor is expressed on the surface of hepatocytes, macrophages, lymphocytes, adrenocortical cells, gonadal cells, and smooth muscle cells. Interaction of apoB100 with the LDL receptor facilitates receptor-mediated endocytosis of LDL particles and subsequent vesicle fusion with a lysosome (Fig. 22-4). The LDL receptor is recycled to the cell surface, while the LDL particle is hydrolyzed to release unesterified cholesterol into the cytoplasm for such cellular functions as cell membrane synthesis and maintenance of cholesterol homeostasis. Three major cholesterol homeostasis pathways are affected by cytoplasmic cholesterol (Fig. 22-4). First, intracellular cholesterol inhibits **HMG CoA reductase,** the enzyme that catalyzes the rate-limiting step in de novo cholesterol synthesis. Second, cholesterol activates acetyl-coenzyme A:cholesterol acyltransferase **(ACAT)** to increase esterification and storage of cholesterol in the cell. Third, cholesterol inhibits **LDL receptor synthesis,** to reduce accumulation of excess cholesterol in the cells. The majority of LDL (70%) is removed from the circulation by the liver. In addition to storage of cholesterol within hepatocytes, the liver serves as the only conduit for cholesterol excretion, via secretion into bile as bile acids or free cholesterol. The sterol nucleus of cholesterol cannot be catabolized.

LDL particles that are not taken up by LDL-R-expressing tissues migrate into the intima of blood vessels and can bind to proteoglycans, where they are oxidized or nonenzymatically glycosylated. Oxidation of LDL results in lipid peroxi-

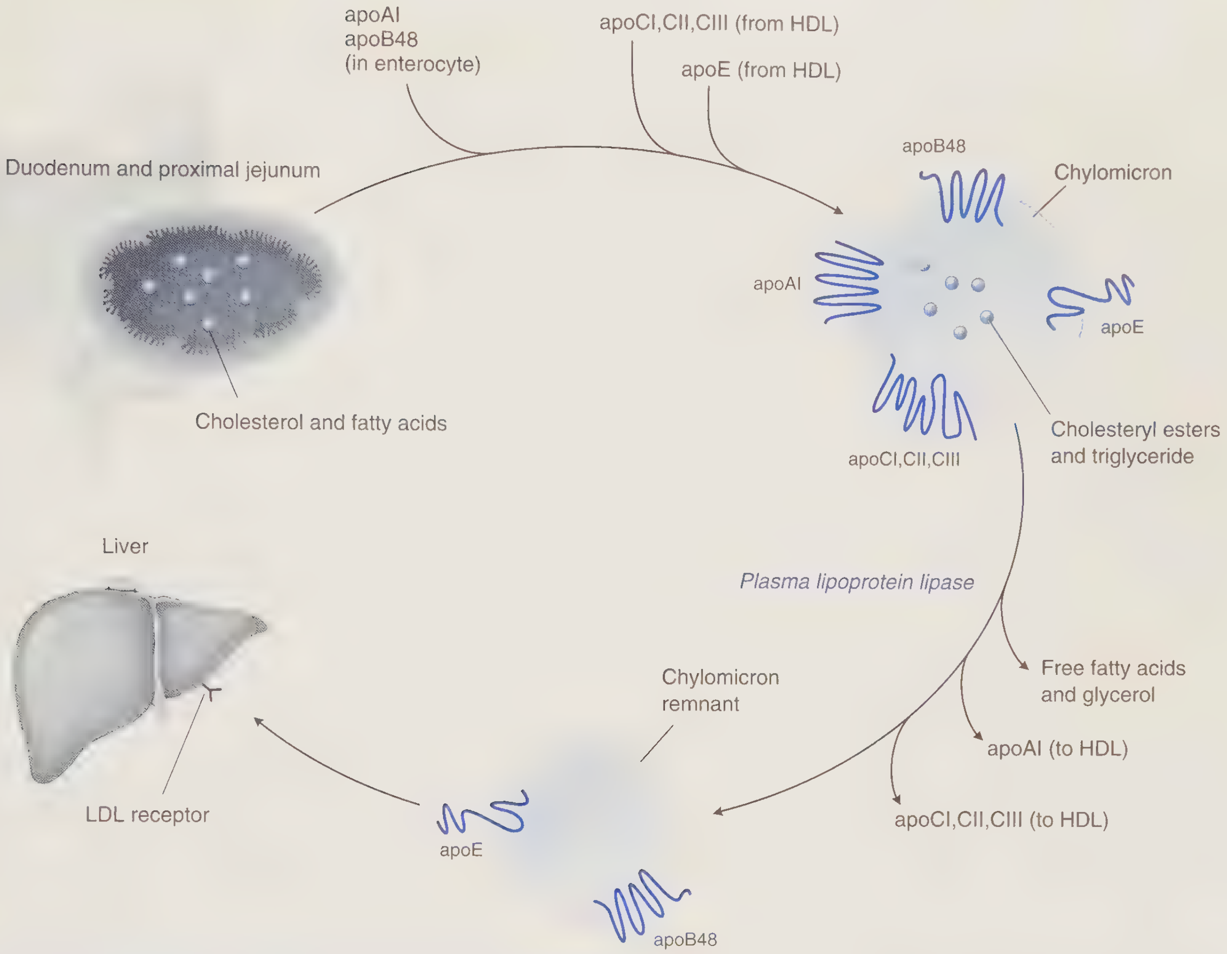

apoAI
apoB48
(in enterocyte)
apoCI,CII,CIII (from HDL)
apoE (from HDL)
Duodenum and proximal jejunum
apoB48
Chylomicron
apoAI
apoE
Cholesterol and fatty acids
apoCI,CII,CIII
Cholesteryl esters and triglyceride
Liver
Plasma lipoprotein lipase
Free fatty acids and glycerol
Chylomicron remnant
apoAI (to HDL)
LDL receptor
apoE
apoCI,CII,CIII (to HDL)
apoB48

**Figure 22-2. Extrinsic (Dietary) Cholesterol and Fatty Acid Transport.** Dietary cholesterol and fatty acids are absorbed by enterocytes in the duodenum and proximal jejunum. These lipids are then esterified and packed into chylomicrons in association with the apolipoproteins apoB48 and apoAI. After entering the circulation through the thoracic duct, chylomicrons acquire the apolipoproteins apoE, apoCI, apoCII, and apoCIII from HDL. Circulating chylomicrons are depleted of triglycerides by the action of lipoprotein lipase, in a reaction that is dependent on apoCII. After lipoprotein lipase has removed a large proportion of the triglyceride core, chylomicrons lose many of their apolipoproteins; the resulting lipoprotein is termed a chylomicron remnant. The liver takes up these remnants in an interaction mediated by apoE binding to the LDL receptor or the LDL-related receptor (not shown).

dation and may create reactive aldehyde intermediates that fragment apoB100. This altered LDL is internalized by the **scavenger receptor** (CD36/SR-A), which is expressed predominantly by mononuclear phagocytic cells. Scavenger receptor-mediated uptake of altered LDL may be an important mechanism for LDL removal (see below), but increased accumulation of oxidized LDL in macrophages can lead to **foam cell** formation (cholesterol-rich macrophages). These foam cells can release oxidized LDL particles, free radicals, and proteolytic enzymes. Foam cells are a major constituent of atherosclerotic plaques, and excessive foam cell apoptosis can destabilize plaques, and hence, predispose to cardiovascular disease. In addition, apoptotic foam cells can destabilize an atherosclerotic plaque. There is also evidence that oxidized LDL can cause up-regulation of cytokines, impair endothelial function, and up-regulate endothelial adhesion molecules. All of these effects cause an increase in the local inflammatory response and promote atherosclerosis (Fig. 22-5). For this reason, *high plasma levels of LDL are a major risk factor for the development of atherosclerosis and subsequent cardiovascular disease;* this is why Jake's doctor became concerned when he discovered that Jake had very high levels of circulating LDL. As a result of the strong correlation between elevated LDL and risk of cardiovascular mortality, the majority of pharmacologic therapies discussed below are targeted at reducing plasma LDL concentration.

## ROLE OF HDL

High density lipoproteins (HDL) are a class of lipoproteins that play a key role in distributing cholesterol, triglycerides, and apolipoproteins between cellular compartments (Fig. 22-6). The major apolipoprotein of HDL is apoAI, although HDL particles are a reservoir for all apolipoproteins except apoB. HDL is synthesized mainly in hepatocytes, although enterocytes also contribute a small percentage to HDL production. Newly formed small HDL particles consist mainly of phospholipid and apolipoprotein AI, with little hydrophobic core. (HDL can also arise from chylomicrons and VLDL during lipolysis; in this case, released phospholipid, cholesterol, and apoAI are combined to form the HDL.)

Because small HDL is relatively cholesterol-poor, free cholesterol equilibrates from cholesterol-rich cells into it.

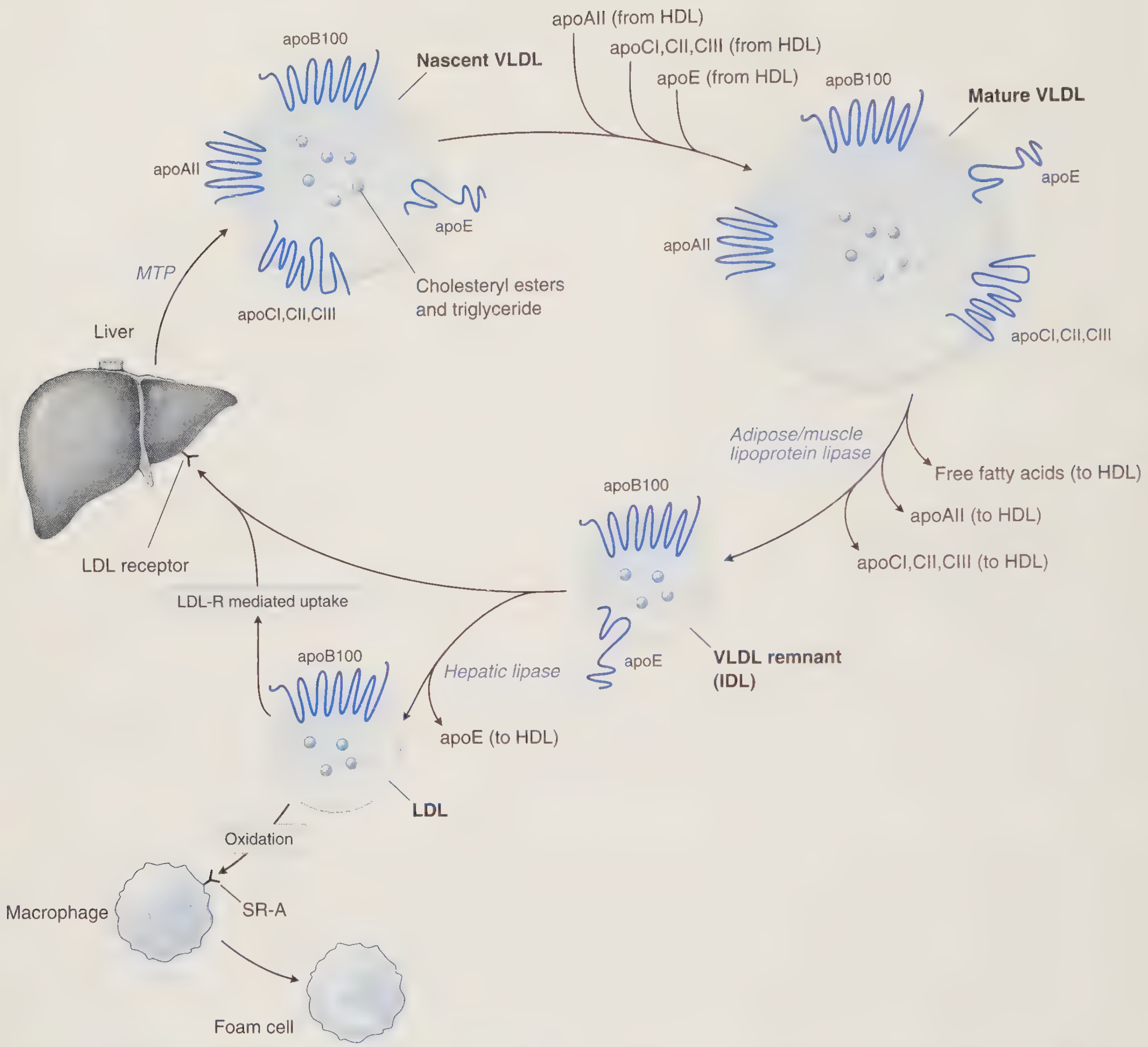

apoB100
Nascent VLDL
apoAII
apoE
apoCI,CII,CIII
Cholesteryl esters and triglyceride
MTP
Liver
apoAII (from HDL)
apoCI,CII,CIII (from HDL)
apoE (from HDL)
apoB100
Mature VLDL
apoE
apoAII
apoCI,CII,CIII
Adipose/muscle lipoprotein lipase
Free fatty acids (to HDL)
apoAII (to HDL)
apoCI,CII,CIII (to HDL)
apoB100
LDL receptor
LDL-R mediated uptake
apoE
VLDL remnant (IDL)
Hepatic lipase
apoB100
apoE (to HDL)
LDL
Oxidation
Macrophage
SR-A
Foam cell

**Figure 22-3. Endogenous Cholesterol and Fatty Acid Transport.** The liver produces nascent VLDL particles by combining triglyceride with apolipoprotein apoB100 (the required structural protein) and other small apolipoproteins including apoE, apoCI, apoCII, apoCIII, and apoAII; this reaction is dependent on microsomal triglyceride transfer protein (MTP). Nascent VLDL particles become mature VLDL as circulating HDL enriches the VLDL with additional apolipoproteins. The triglyceride core of VLDL is removed by the action of lipoprotein lipase on the endothelial cells of adipose and muscle tissue. Depletion of the triglyceride core results in the formation of VLDL remnants (IDL and LDL). IDL can be removed from the circulation via LDL receptor (LDL-R)-mediated uptake, or hepatic lipase can hydrolyze the remaining triglycerides, resulting in the formation of LDL. LDL is removed from the circulation by LDL-R-mediated uptake by the liver or other tissues. Alternatively, LDL can be oxidized and taken up by macrophages, in a reaction that depends on scavenger receptor-A (SR-A); this reaction results in the formation of foam cells. Note the many points of intersection between HDL and endogenous lipid metabolism.

Cellular cholesterol efflux is dependent on the **ABCA1** (ATP-Binding Cassette transporter A1) gene product, an ATP-binding transmembrane protein that catalyzes transport of cholesterol from intracellular stores to the cell surface, and then to HDL. Once cholesterol is transferred from the cell surface to HDL, the HDL apolipoprotein apoAI acts as a cofactor for the plasma enzyme lecithin:cholesterol acyltransferase **(LCAT),** which esterifies cholesterol; the cholesteryl ester product forms the hydrophobic core of HDL. As HDL accumulates more cholesterol from tissues, it becomes larger.

Three mechanisms exist for HDL-mediated redistribution of cholesterol. First, interaction of HDL with the HDL receptor **SR-BI,** which is present in the liver, adrenal glands, and gonads, mediates transfer of cholesterol from HDL to these tissues. Second, HDL equilibrates cholesterol between lipoproteins via **cholesterol ester transfer protein (CETP),** resulting in a net efflux of cholesterol from HDL to VLDL and IDL, and an increase in the triglyceride content of HDL. Third, **hepatic lipase,** on the hepatocyte surface, hydrolyzes triglyceride and cholesteryl ester in HDL, forming small HDL, which then recirculates in plasma. The "lifespan" of an HDL particle is 2–5 days, suggesting that each HDL particle can have many cycles of cholesterol absorption and delivery to the liver.

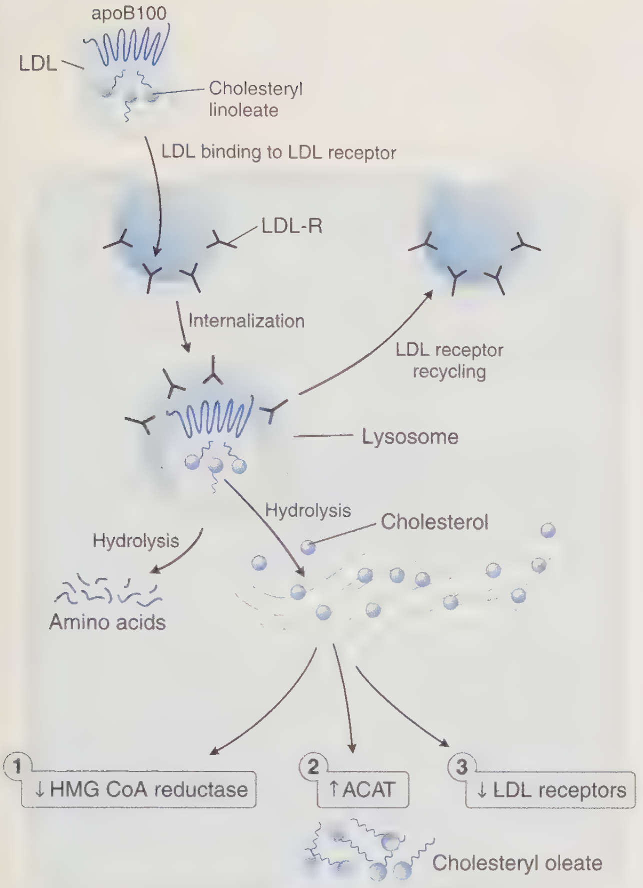

apoB100
LDL
Cholesteryl linoleate
LDL binding to LDL receptor
LDL-R
Internalization
LDL receptor recycling
Lysosome
Hydrolysis
Hydrolysis
Cholesterol
Amino acids
1 ↓ HMG CoA reductase
2 ↑ ACAT
3 ↓ LDL receptors
Cholesteryl oleate

**Figure 22-4. Cellular Regulation of Cholesterol Metabolism.** Binding of apolipoprotein B100 to LDL receptors (LDL-R) promotes LDL internalization into endocytic vesicles and fusion of the vesicles with lysosomes. LDL-R is recycled to the cell surface, while the lipoprotein particle is hydrolyzed into amino acids and free cholesterol. Intracellular cholesterol has three regulatory effects on the cell. First, cholesterol decreases the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Second, cholesterol activates acetyl CoA:cholesterol acyltransferase (ACAT), an enzyme that esterifies free cholesterol into cholesteryl ester (cholesteryl oleate) that can be stored in the cell. Third, cholesterol inhibits the transcription of the gene encoding the LDL receptor, and, thereby, decreases further uptake of cholesterol by the cell.

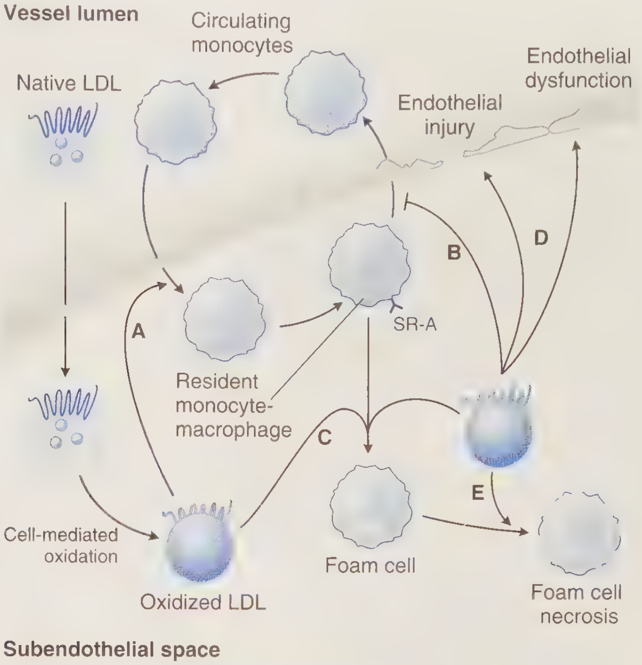

Vessel lumen
Circulating monocytes
Native LDL
Endothelial dysfunction
Endothelial injury
A
B
C
D
E
SR-A
Resident monocyte-macrophage
Cell-mediated oxidation
Oxidized LDL
Foam cell
Foam cell necrosis
Subendothelial space

**Figure 22-5. LDL and Atherosclerosis.** Elevated LDL is a major risk factor for the development of atherosclerosis. Native LDL that migrates into the subendothelial space can undergo chemical transformation to oxidized LDL via lipid peroxidation and fragmentation of apoB100. Oxidized LDL has a number of deleterious effects on vascular function. Oxidized LDL promotes monocyte chemotaxis into the subendothelial space **(A)** and inhibits monocyte egress from that space **(B).** Resident monocyte–macrophages bind to oxidized LDL via a scavenger receptor (SR-A), resulting in the formation of lipid-laden foam cells **(C).** Oxidized LDL can directly injure endothelial cells and cause endothelial dysfunction **(D).** Oxidized LDL can also cause foam cell necrosis, with release of numerous proteolytic enzymes that can damage the intima **(E).**

The net result of this cycle is removal and redistribution of lipids—a process referred to as **reverse cholesterol transport.** Recall from Figure 22-5 that oxidation of LDL and subsequent uptake of oxidized LDL by macrophages is an initiating event in atherosclerosis. Because HDL removes cholesterol from macrophages and promotes the return of cholesterol to hepatic sites, HDL decreases the extent of cholesterol available for deposition in tissues. Increased levels of HDL may further decrease the amount of cholesterol deposition; for this reason, there exists an inverse relationship between plasma HDL levels and risk of cardiovascular disease. HDL also has direct beneficial effects on vascular tissue, including enhancement of antioxidant enzyme activities that inhibit oxidation of LDL. HDL also inhibits the expression of inflammatory mediators by vascular cells (for example, ICAM and VCAM by endothelial cells). Increased understanding of these mechanisms may lead to the development of novel biochemical targets for increasing reverse cholesterol transport and, thus, potentially slowing or reversing the progression of atherosclerosis.

## PATHOPHYSIOLOGY

Numerous studies have demonstrated a definitive link between elevated plasma levels of cholesterol and long-term risk of cardiovascular disease. This increased risk of cardiovascular mortality is most closely linked to increased levels of LDL and decreased levels of HDL, but hypertriglyceridemia is also an independent risk factor. When LDL levels are low, high triglyceride and low HDL are increasingly important as risk factors. From a clinical perspective, the dyslipidemias can be divided into hypercholesterolemia, hypertriglyceridemia, mixed hyperlipidemia, and disorders of HDL metabolism (Table 22-3).

Numerous causes of hyperlipidemia exist. These include well-defined monogenic conditions, genetic polymorphisms, and less well defined gene–environment interactions. For many individuals, elevated cholesterol may be the result of a diet high in cholesterol and saturated fat superimposed on a susceptible genetic profile. The following section presents the major defined genetic predispositions for hyperlipidemia,

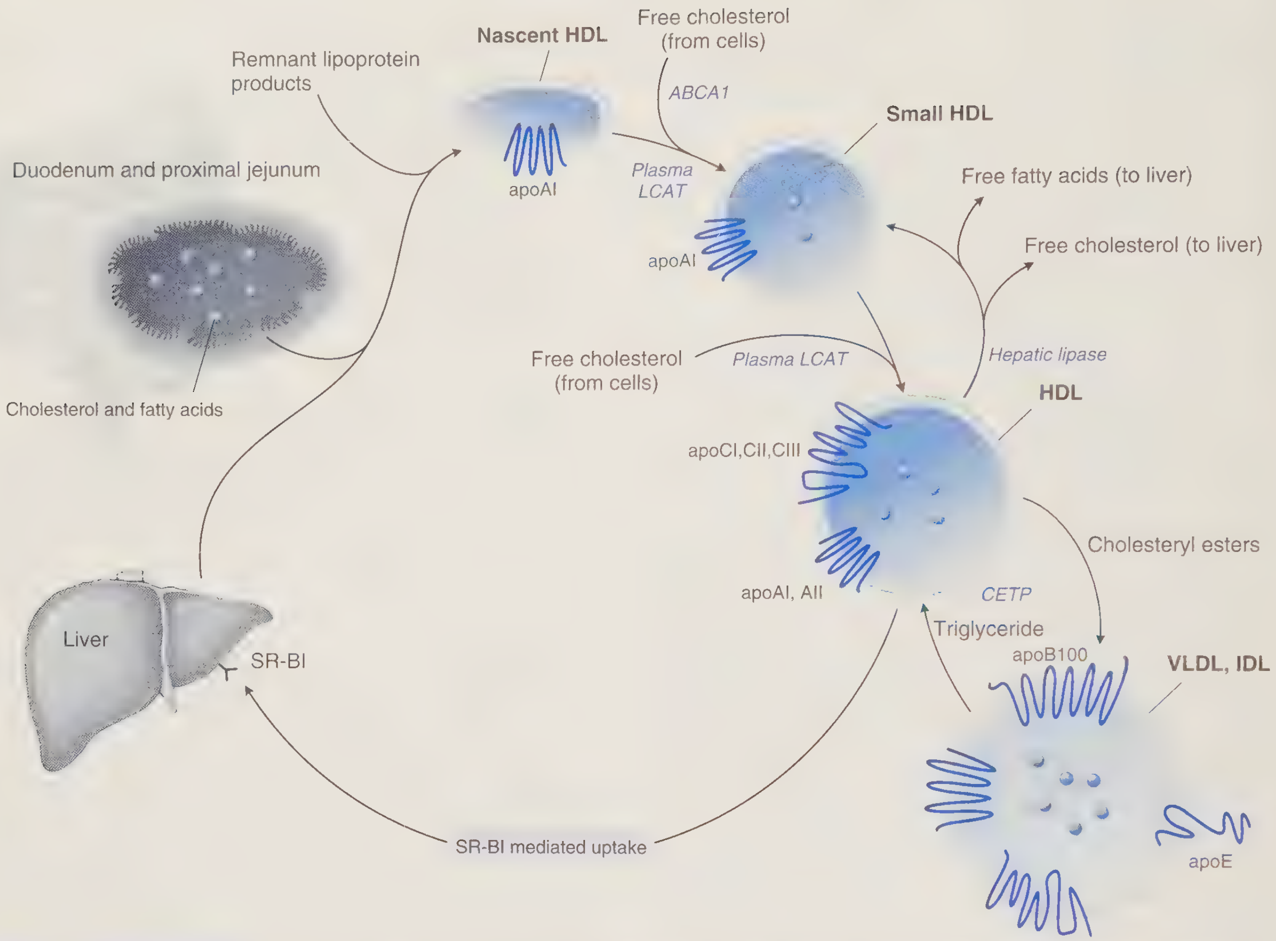

Nascent HDL
Free cholesterol (from cells)
Remnant lipoprotein products
ABCA1
Small HDL
Duodenum and proximal jejunum
apoAI
Plasma LCAT
Free fatty acids (to liver)
apoAI
Free cholesterol (to liver)
Free cholesterol (from cells)
Plasma LCAT
Hepatic lipase
HDL
Cholesterol and fatty acids
apoCI,CII,CIII
Cholesteryl esters
apoAI, AII
CETP
Triglyceride
Liver
apoB100
VLDL, IDL
SR-BI
SR-BI mediated uptake
apoE

**Figure 22-6. HDL Metabolism.** HDL originates in the liver or the intestine or from remnant lipoprotein products released during the hydrolysis of lipoproteins by plasma lipoprotein lipase. Nascent HDL is cholesterol poor; it contains mainly phospholipid and the apolipoprotein apoAI. Nascent HDL circulates in the plasma and receives free cholesterol from cholesterol-laden cells, including macrophages, by a process that is dependent on the enzyme ATP-binding cassette transporter A1 (ABCA1). Using apoAI as a cofactor, plasma lecithin:cholesterol acyl transferase (LCAT) esterifies the free cholesterol, resulting in a larger hydrophobic core of cholesteryl esters (small HDL). HDL becomes larger as it accumulates more cholesteryl esters. HDL can transfer cholesteryl esters to VLDL and IDL via the enzyme cholesteryl ester transfer protein (CETP) and accumulate triglyceride from the VLDL and IDL. The relatively triglyceride-rich HDL can then be eliminated by one of three mechanisms. First, HDL can be taken up directly by the hepatic SR-B1 receptor. Second, hepatic lipase can hydrolyze the triglyceride core, regenerating small HDL. Third, HDL can acquire the apolipoprotein apoE; this particle can then be taken up by the hepatic LDL-R (not shown).

followed by a brief overview of the secondary causes of hyperlipidemia. It should be noted that, in a clinical setting, the decision to treat elevated cholesterol levels is based on risk of cardiovascular disease, not on identification of the defined genetic cause of hyperlipidemia. As common genetic predispositions to dyslipidemia become better elucidated, lipid-lowering therapies may one day be tailored toward individual genetic susceptibilities.

## HYPERCHOLESTEROLEMIA

Primary hypercholesterolemia is characterized by elevated levels of total plasma cholesterol and LDL cholesterol, and normal levels of triglyceride, causing elevated risk for atherosclerosis and cardiovascular disease. The causes of primary hypercholesterolemia are familial hypercholesterolemia, familial defective apoB100 and, most commonly, polygenic hypercholesterolemia.

Familial hypercholesterolemia (FH) is an autosomal dominant disease involving defects in the high-affinity LDL receptor (LDL-R). Mutations in the gene encoding the receptor include lack of receptor production, failure of receptors to reach the cell membrane, receptors with defective LDL binding, and internalization defects in which plasma membrane LDL receptors are not localized in regions of coated pits and, thus, do not undergo endocytosis when bound to LDL particles. Heterozygous individuals (1 in 500 in the U.S.) have elevated plasma cholesterol levels from birth throughout life, with average adult levels of 275–500 mg/dL (normal, <200 mg/dL). Clinical features include tendon xanthomas (caused by intracellular and extracellular accumulation of cholesterol) and arcus corneae (deposition of cholesterol in the cornea). Homozygous FH is a more severe but rare disorder

**TABLE 22-3** Genetic Causes of Dyslipidemia

| DISEASE | CHARACTERISTIC LIPID PROFILE | ESTIMATED PREVALENCE | ETIOLOGY |
|---|---|---|---|
| **Primary Hypercholesterolemia** | | | |
| Familial hypercholesterolemia | ↑↑ LDL | 1:500 (heterozygote)<br>1:1 million (homozygote) | ↓/No functional LDL-R expression |
| Familial defective apoB100 | ↑ LDL | 1:1,000 | ↓ Binding of apoB100 to LDL-R |
| Polygenic hypercholesterolemia | ↑ Cholesterol | Common | Unknown. Variants in genes for lipid metabolism increasing susceptibility to diet |
| **Primary Hypertriglyceridemia** | | | |
| Familial hypertriglyceridemia | ↑ TGs, ↑ VLDL, ↓ HDL | Common | Overproduction and impaired catabolism of triglyceride-rich VLDL |
| Familial lipoprotein lipase deficiency | ↑↑ TGs | 1:1 million | Defect in lipoprotein lipase |
| ApoCII deficiency | ↑↑ TGs | 1:1 million | Defect in apoCII |
| **Mixed Hyperlipidemia** | | | |
| Familial combined hyperlipidemia | ↑ LDL, ↑TGs, sometimes ↓ HDL | 1:100 | Unknown. Dominant inheritance. |
| Familial dysbetalipoproteinemia | ↑ Cholesterol, ↑ TGs, ↓ LDL, ↑ remnants | 1:10,000 | Inheritance of apoE2 isoform |
| **Disorders of HDL Metabolism** | | | |
| Polygenic low HDL | ↓ HDL | Common | Overweight, diabetes, lack of exercise, high carbohydrate diet |
| Familial hypoalphalipoproteinemia | ↓ HDL | 1:400 | Unknown. Dominant inheritance |
| Familial apoAI deficiency | ↓ HDL | Rare | ApoAI |
| Tangier disease | ↓↓ HDL | Rare | ABCA1 defect |
| LCAT deficiency | ↓ HDL | Rare | LCAT |
| Fisheye disease | ↓ HDL | Rare | Low activity of LCAT |
| CETP deficiency | ↑ HDL | Rare | CETP |

Dyslipidemias can be categorized by increases in plasma cholesterol, increases in triglycerides, increases in both cholesterol and triglycerides, or disturbances in HDL metabolism. The genetics of the common causes of dyslipidemia are currently largely unknown and may be ascribed to polygenic risk factors or unknown genetic susceptibilities to diet and lifestyle. (See Tables 22-1 and 22-2 for abbreviations).

(1 in 1 million in the U.S.) characterized by absence of functional LDL receptors. This leads to very high plasma cholesterol (700–1,200 mg/dL) and cardiovascular disease before age 20. Heterozygotes for FH respond well to statins and other LDL-lowering drugs; Jake likely was heterozygous for FH. Because homozygotes lack functional receptor, the only effective treatment is plasmapheresis with immunoadsorption of LDL.

Familial defective apoB100 is an autosomal dominant trait in which mutations in the apoB100 protein lead to decreased affinity of the LDL particle for LDL receptors and, thus, to decreased catabolism of LDL. Cholesterol levels can be similar to those in patients with FH. These individuals respond well to therapy with a statin and niacin.

Polygenic hypercholesterolemia is a general term used to categorize the >85% of patients with hypercholesterolemia who have no defined genetic cause. Polygenic hypercholesterolemia may be the result of complex gene–environment interactions and/or multiple uncharacterized genetic susceptibilities. Further research into genetic predispositions for hypercholesterolemia will be necessary before a clear etiology can be identified for the majority of patients with hypercholesterolemia.

## HYPERTRIGLYCERIDEMIA

Primary hypertriglyceridemia is characterized by high plasma triglyceride levels (200–500 mg/dL; normal, <150 mg/dL) after an overnight fast. The three major etiologies of hypertriglyceridemia that have been identified are familial hypertriglyceridemia of unknown genetic cause, familial lipoprotein lipase deficiency, and apoCII deficiency. Commonly, hypertriglyceridemia develops with age and from weight gain, obesity, and diabetes.

Familial hypertriglyceridemia is a common autosomal dominant disorder characterized by hypertriglyceridemia with normal LDL levels. HDL is often reduced. The underlying defect in this disorder is unknown, but is postulated to be enhanced hepatic triglyceride synthesis leading to increased triglyceride levels in VLDL, and increased production of VLDL. Decreased lipolysis of chylomicrons and VLDL by lipoprotein lipase is another cause. Fibrates are the drug of choice for familial hypertriglyceridemia; niacin and statins may be added to this regimen if needed.

Familial lipoprotein lipase deficiency is an autosomal recessive trait caused by absence of active lipoprotein lipase. This leads to profound hypertriglyceridemia, which is characterized by elevated chylomicrons during infancy and impaired removal of VLDL later in life. Infants or young adults can present with pancreatitis, eruptive xanthomas, hepatomegaly, and splenomegaly because of accumulation of lipid-laden foam cells. Treatment consists of a fat-free diet and avoidance of substances that increase VLDL, such as alcohol and glucocorticoids.

ApoCII deficiency is a rare autosomal disorder with presentation and treatment similar to familial lipoprotein lipase deficiency. Differentiation between these two disorders can be performed by demonstrating that transfusion with apoCII reduces triglyceride levels in patients with apoCII deficiency, but not in patients with familial lipoprotein lipase deficiency.

## MIXED HYPERLIPIDEMIA

Patients with mixed hyperlipidemia possess complex lipid profiles that may consist of elevated total cholesterol, elevated LDL, and increased triglycerides. HDL is often reduced. Two specific etiologies of mixed hyperlipidemia are familial combined hyperlipidemia (FCH) and dysbetalipoproteinemia.

FCH is a common disease associated with moderately elevated levels of fasting triglycerides and total cholesterol but reduced HDL. These patients often present with central obesity, hyperinsulinemia, glucose intolerance, and mild hypertension. The molecular cause is still under investigation, but current hypotheses include increased synthesis of VLDL, leading to increased apoB levels; defective LDL receptors; or low levels of lipoprotein lipase. (It is likely that a combination of several genetic variants is involved.) Dietary modification, if adhered to faithfully, may be an effective means of controlling FCH, but drug treatments are often required. Statins are commonly employed; combination therapy with a fibrate or niacin may be necessary to normalize both LDL and triglyceride levels, as well as to increase HDL.

Dysbetalipoproteinemia is a disorder characterized by increased cholesterol-rich chylomicrons and IDL-like particles. These findings result from accumulated chylomicron and VLDL remnants, leading to both hypertriglyceridemia and hypercholesterolemia. Mutations in isoforms of apoE (E2, E3, and E4) have been implicated in the disease. Patients with the homozygous apoE2/apoE2 phenotype have reduced chylomicron and VLDL affinity for lipoprotein receptors, leading to accumulation of remnant particles in the plasma. Although the defective apoE is present at birth, symptoms generally do not present in females until menopause and in males until age 30. The reason for this is unknown, but it is suspected that additional metabolic factors (such as obesity, diabetes, or hypothyroidism) may be required to unmask the disorder. Decreased intake of fat and cholesterol, along with weight reduction and omission of alcohol intake, can effectively manage the disorder. In addition, niacin and fibrates are effective pharmacologic therapies.

## DISORDERS OF HDL METABOLISM

Decreased HDL level is an independent risk factor for development of atherosclerosis and cardiovascular disease. Numerous rare genetic defects in HDL metabolism have been identified, including defects in apoAI, ABCA1, and LCAT. All of these defects result in decreased levels of HDL, either from decreased HDL production (as in apoAI deficiency) or increased HDL catabolism (as in Tangier disease). No effective treatments currently exist for these diseases.

Recently, variations in CETP activity have also been characterized as a potentially more common source of interindividual variation in HDL levels. Decreased CETP activity results in increases in plasma HDL concentration, attributable to less transfer of cholesterol from HDL to VLDL. Although it might be assumed that the increased HDL levels would be cardioprotective, this is not always observed; in some cases, decreased CETP activity may increase the risk of atherogenesis; whereas, in others, it may be cardioprotective. This observation may be attributable to an alteration in the quality of the increased HDL, so that the resulting HDL is not as effective at removing lipids. Elucidation of the physiologic role of CETP has also been confounded by conflicting results of animal studies; further research will be necessary before the role of CETP polymorphisms in lipid metabolism and cardiovascular disease risk can be identified.

## SECONDARY HYPERLIPIDEMIA

In addition to the genetic causes of primary dyslipidemia detailed above, a number of secondary factors can lead to hyperlipidemia (Table 22-4). For example, alcohol intake is known to increase NADH activity in the liver, leading to an

**TABLE 22-4** Secondary Causes of Hyperlipidemia

| HYPERTRIGLYCERIDEMIA | HYPERCHOLESTEROLEMIA |
|---|---|
| Diabetes mellitus | Hypothyroidism |
| Chronic renal failure | Nephrotic syndrome |
| Hypothyroidism | Anorexia nervosa |
| Glycogen storage disease | Acute intermittent porphyria |
| Stress | Cholestasis |
| Sepsis | Obstructive liver disease |
| Alcohol excess | Corticosteroid treatment |
| Lipodystrophy | Protease inhibitor therapy |
| Pregnancy | |
| Oral estrogen replacement therapy | |
| Antihypertensive drugs: beta-blockers, diuretics | |
| Glucocorticoid treatment | |
| Protease inhibitor therapy | |
| Acute hepatitis | |
| Systemic lupus erythematosus | |

Numerous secondary causes of hyperlipidemia exist; screening for the presence of these underlying factors should be performed before initiating pharmacologic therapy for a dyslipidemia.

increase in the production of fatty acids, which are then esterified to glycerol to form triglycerides. Moderate alcohol consumption can, therefore, result in increased VLDL. Hypertriglyceridemia in noninsulin-dependent diabetes results from increased VLDL synthesis and reduced chylomicron and VLDL catabolism by LPL. Insulin normally suppresses VLDL production by the liver; thus, insulin resistance in the liver causes increased VLDL production. Furthermore, apoCIII levels are increased, perhaps because of an insulin response element in the apoCIII gene. High apoCIII reduces the catabolism of chylomicrons and VLDL.

Hypothyroidism is an important and common cause of secondary hyperlipidemia. All patients with any lipid disorder should be screened for hypothyroidism with a TSH determination.

## PHARMACOLOGIC CLASSES AND AGENTS

The decision to treat dyslipidemia is dependent on the extent of lipid elevation and calculated cardiovascular risk. A number of clinical algorithms exist for determining initiation of therapy; the most recent (National Cholesterol Education Program Adult Treatment Panel III [ATP III]) provides various target LDL levels based on 10-year risk of death from cardiovascular disease (Table 22-5). Once the decision to treat has been made, it is important first to promote therapeutic lifestyle changes (TLC), which include reduction of saturated fat and cholesterol intake, weight reduction, increased physical activity, and perhaps stress reduction.

Successful dietary therapy can reduce total cholesterol by 5–25% depending on adherence. If this approach is unsuccessful or insufficient to normalize lipid levels, drug therapy is recommended. Pharmacologic modification of lipid metabolism employs five classes of agents. Three of these classes, bile acid sequestrants, inhibitors of cholesterol absorption, and HMG CoA reductase inhibitors, have well-defined effects on lipid metabolism. The other two classes, fibrates and niacin, have more wide-ranging effects on lipid metabolism. Although the overall metabolic effects of fibrates and niacin are well understood, their molecular actions are still active areas of investigation. Statins are the most important class of agents because of their demonstrated efficacy in both primary and secondary prevention of cardiovascular disease morbidity and mortality. However, the other classes all act as important adjunctive therapies and are the agents of choice for patients with certain specific causes of dyslipidemia.

### INHIBITORS OF BILE ACID ABSORPTION

The bile acid sequestrants are cationic polymers that bind noncovalently to negatively charged bile acid molecules in the small intestine. The sequestrant–bile acid complex cannot be absorbed and is excreted into the stool. Decreased bile acid reabsorption by the ileum interrupts enterohepatic bile acid circulation, causing hepatocytes to up-regulate 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis from cholesterol. *The increased bile acid synthesis decreases hepatocyte cholesterol concentration, leading to increased expression of LDL-R and, thus, enhanced LDL clearance from the circulation;* this is thought to be the primary mechanism by which bile acid sequestrants lower plasma LDL. The plasma clearance of LDL is partially offset by concurrent hepatocyte up-regulation of HMG CoA reductase and triglyceride synthesis, which stimulate endogenous cholesterol synthesis and VLDL production.

**TABLE 22-5** National Cholesterol Education Program Adult Treatment Panel III Guidelines

| RISK CATEGORY | INITIATION LDL LEVEL FOR DRUG TREATMENT | LDL GOAL |
|---|---|---|
| High: CHD or CHD risk equivalents (10-year risk > 20%) | 130 mg/dL (100 mg/dl optional) | <100 mg/dL |
| Moderate: 10-yr risk 10%–20% | 130 mg/dL | <130 mg/dL |
| Moderate: 10-yr risk <10% and 2 or more risk factors | 160 mg/dL | <130 mg/dL |
| Low: 0–1 risk factor | 190 mg/dL (160 mg/dl optional) | <160 mg/dL |

The decision to initiate lipid-lowering therapy depends on the risk category for cardiovascular disease or equivalents (peripheral vascular disease, abdominal aortic aneurysm, cerebrovascular disease, or diabetes). A patient with documented cardiovascular disease or equivalent risk (>20% in 10 years) should have target LDL levels <100 mg/dL. A patient with two or more risk factors (cigarette smoking, hypertension, low HDL, positive family history of CAD, male >45 years, female >55 years) has moderate risk of mortality from cardiovascular disease, and LDL levels should be modified to <130 mg/dL. Subtract one risk factor when HDL is high (>60 mg/dL). Patients with 0–1 risk factors have a low risk of cardiovascular disease, and their LDL levels should be maintained at <160 mg/dL with either diet or drug therapy (drug therapy is indicated by an LDL level, after nonpharmacologic therapy of >190 mg/dL).

The two available bile acid sequestrants are **cholestyramine and colestipol.** They possess similar efficacy, with an average 8–24% reduction in LDL levels at therapeutic concentrations. Bile acid sequestrants may also raise triglyceride levels because of increased hepatocyte triglyceride synthesis. For this reason, bile acid sequestrants should be used with caution in patients with hypertriglyceridemia.

Because bile acid sequestrants are not absorbed systemically, they have little potential for serious toxicity. However, significant bloating and dyspepsia often limit patient compliance; these side effects result from decreased fat absorption and bile acid irritation of the large intestine. Because these drugs interrupt lipid absorption, they can also decrease absorption of fat-soluble vitamins; bleeding as a result of vitamin K deficiency secondary to bile acid sequestrant use has been reported occasionally. The bile acid sequestrants can also bind certain drugs, such as digoxin and warfarin; this drug interaction can be eliminated by administering the bile acid sequestrant at least 1 hour after other drugs.

Because of the demonstrated clinical efficacy and tolerability of statins, bile acid sequestrants have been relegated to second-line agents for lipid reduction. Currently, bile acid sequestrants are used mainly for treatment of hyperlipidemia in young (<25 years old) patients and in patients for whom statins alone do not provide sufficient plasma LDL reduction. Some experts prefer bile acid sequestrants for young patients (such as patients with familial hypercholesterolemia) because these agents are not absorbed and are generally considered safe for long-term use; whereas, other experts prefer to use a statin for initial therapy in children. For patients who require more aggressive lipid lowering than is possible with statins alone, co-administration of a statin with a bile acid sequestrant has an additive effect on lipid reduction.

## INHIBITORS OF CHOLESTEROL ABSORPTION

**Colesevelam** is a synthetic soluble fiber that, at therapeutic doses, reduces LDL cholesterol by 10%–16%. Colesevelam is not absorbed from the intestine, and the incidence of side effects is similar to placebo. Six tablets of colesevelam must be taken per day; the size and bulk of this dosing pattern is inconvenient for some patients and, therefore, limits compliance.

**Ezetimibe** is an inhibitor of intestinal absorption of cholesterol. A single daily dose lowers LDL cholesterol by about 14%, an effect that is additive to the effects of statins in typical patients with mild elevation of LDL. Patients with familial hypercholesterolemia may experience greater LDL reduction. Ezetimibe produces similar additional LDL reduction throughout the statin dose range. Unlike colesevelam and the bile acid sequesterants (which are not absorbed), ezetimibe is rapidly absorbed by the enterocyte and extensively glucuronidated, and systemic concentrations of both unmodified and glucuronidated forms can be measured. Ezetimibe recirculates enterohepatically several times per day in coordination with meals, and the systemic concentration of ezetimibe also fluctuates throughout the day. Ezetimibe may act inside the enterocyte and not in the intestinal lumen; the molecular mechanism of action is unknown. Ezetimibe has little effect on HDL cholesterol and triglyceride levels. Ezetimibe does not cause any known adverse effects or drug interactions.

## INHIBITORS OF CHOLESTEROL SYNTHESIS

HMG CoA reductase inhibitors, commonly known as statins, competitively inhibit the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Inhibition of this enzyme results in an inability of hepatocytes to increase cholesterol synthesis and decreases hepatocyte cholesterol concentration (Fig. 22-7). Decreased cellular cholesterol activates a cellular signaling cascade culminating in the activation of sterol regulatory element binding proteins (SREBPs), which are transcription factors that activate transcription of the gene encoding LDL-R. As with the bile acid sequestrants, the increased expression of LDL-R causes increased uptake of plasma LDL and, consequently, decreases plasma LDL concentrations. The amount of LDL lowering is dependent on drug efficacy, and ranges from 25% to 55%. Statins also modestly raise plasma HDL by an average of 5%, and reduce plasma triglyceride levels by 10%–25% depending on dose and degree of hypertriglyceridemia. The latter effect is caused by decreased VLDL synthesis and increased clearance of lipoproteins by the liver. The dose effect of statins is nonlinear—the largest effect occurs with the starting dose, and then each subsequent doubling of the dose produces, on average, an additional 6% LDL reduction.

In addition to inhibition of HMG CoA reductase, statins may possess a number of other pharmacologic effects, collectively referred to as **pleiotropic effects.** These include reversal of endothelial dysfunction, decreased coagulation, decreased inflammation, and improved plaque stability. Evidence for reversal of endothelial dysfunction is provided by improved vasodilatory response of endothelium to NO after statin administration; the improved vasodilation may help prevent ischemia. Decreased coagulation is supported by decreased prothrombin activation and decreased endothelial cell tissue factor production during statin therapy. Because thrombus development causes most acute coronary syndromes, this effect may account for some of the mortality benefit of statins. Evidence for decreased inflammation during statin therapy includes decreases in acute-phase reactants (plasma proteins that are increased during inflammatory states) such as C-reactive protein; because atherosclerosis is an inflammatory process, the decreased inflammation may retard the progression of atherosclerotic plaques. Plaques also become more stable during statin therapy because of a thicker fibrous cap; this may result from decreased macrophage proliferation and inhibition of vascular smooth muscle proliferation, and may be caused, in part, by the lipid-lowering effects of statins. It should be emphasized that many of these actions have been demonstrated only in vitro or in animal models, and have, therefore, not yet been proved to be major actions of statins in humans. Indeed, analysis of the results of clinical trials of statins attribute most of the cardiovascular disease benefit to LDL lowering.

Statins have been shown in numerous clinical trials to reduce mortality significantly after a myocardial infarction (so-called secondary prevention). For example, in the LIPID trial that included patients with a previous myocardial infarction or unstable angina, pravastatin reduced the risk of coronary mortality by 24%, and the risk of death from any cause by 22%, relative to placebo. Recent studies have also concluded that lowering of LDL with statins can decrease mortality even in the absence of overt cardiovascular disease (primary prevention). Despite these convincing percentage risk reductions

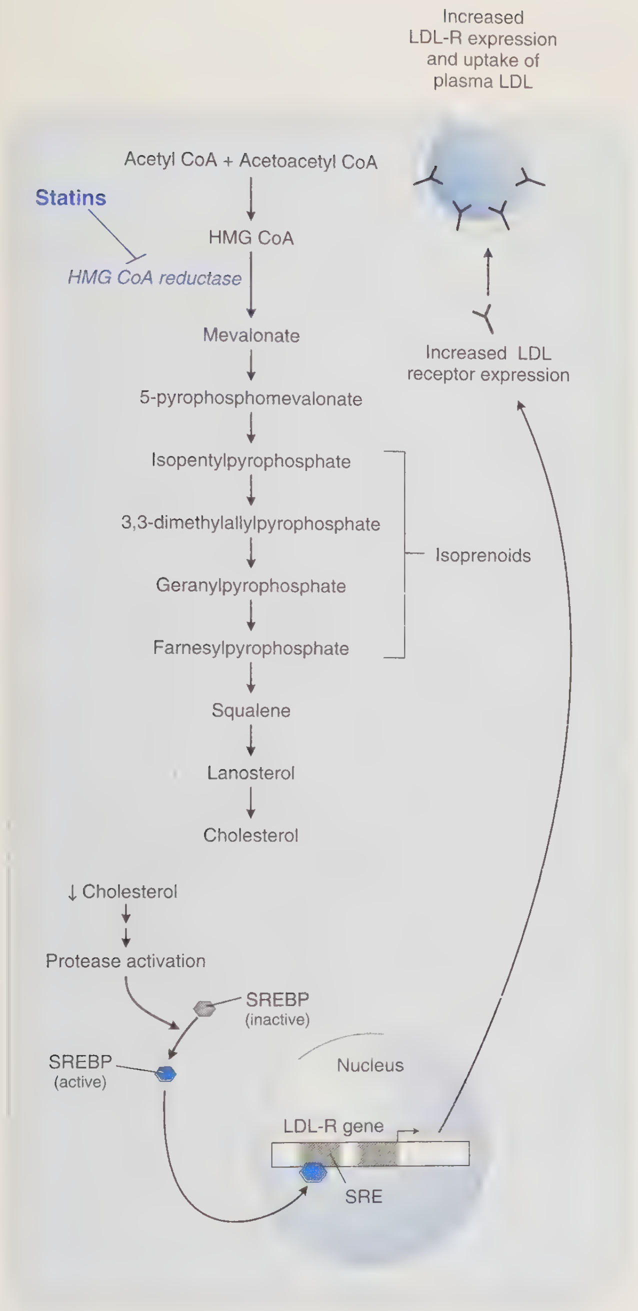


**Figure 22-7. Effects of Statins on Cholesterol Metabolism.** Statins competitively inhibit HMG CoA reductase, the enzyme that catalyzes a crucial step in cholesterol synthesis. Decreased hepatocyte cholesterol concentration leads to protease activation and cleavage of the sterol regulatory element binding protein (SREBP), which is a transcription factor that normally resides in the cytoplasm. The cleaved SREBP diffuses into the nucleus, where it binds to sterol response elements (SRE), leading to up-regulation of LDL-receptor (LDL-R) gene transcription. This leads to increased hepatocyte LDL-R expression, which mediates increased plasma LDL clearance and results in reduced circulating LDL levels.

in both secondary and primary prevention trials, it should be noted that statins create a greater absolute risk reduction in secondary prevention; patients in this treatment group have a greater absolute risk of death and may, therefore, benefit more from statins. Statins have demonstrated reductions in CVD in patients at high risk who have average, or even below-average, LDL levels.

Six statins—**lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin,** and **rosuvastatin**—are currently approved for use in hypercholesterolemia and mixed dyslipidemia. They are considered first-line therapy for increased LDL levels, and numerous trials have shown that statins decrease both cardiovascular-related and total mortality in primary and secondary prevention trials. Stroke is also reduced. All agents are believed to act by the same mechanism of action, and their main differences are attributable to potency and pharmacokinetic parameters. Among the statins, fluvastatin is the least potent, and atorvastatin and rosuvastatin are the most potent; the clinical relevance, if any, of these potency differences has not yet been determined. The pharmacokinetic differences among the statins result from differential P450 metabolism. Lovastatin, simvastatin, and atorvastatin are metabolized by CYP3A4, while other P450-mediated pathways metabolize fluvastatin. Pravastatin is not metabolized via the P450 pathway. Rosuvastatin was approved by the FDA in August 2003. Metabolism has important implications for drug interactions (see below).

Statins are generally well tolerated and have a lower incidence of side effects than any of the other lipid-lowering agents. The main side effect of statins is myopathy and/or myositis with rhabdomyolysis. This is a very rare complication that occurs primarily at the highest doses, e.g., 80 mg of lovastatin or simvastatin. In fact, reports of myalgia and myopathy were similar for pravastatin 40 mg or simvastatin 40 mg compared to placebo in trials of about 20,000 patients. For this reason, plasma creatine kinase levels are not useful for routine monitoring of statin-treated patients.

If a statin alone is insufficient to lower LDL to target levels, statins can be used effectively in combination with other agents. The combination of a statin with a bile acid sequestrant or inhibitor of cholesterol absorption results in additive LDL decreases and lacks significant drug interactions. The combination of niacin and a statin may be most useful in patients with high LDL and low HDL. However, co-administration of niacin and statins slightly increases the risk of myopathy, so this potential side effect should be carefully monitored. Fibrate and statin combinations have also been reported to be efficacious in combination. However, certain fibrates inhibit the glucuronidation of statins; by decreasing statin clearance, these agents raise the plasma concentration of statins. This effect has been documented for gemfibrozil but does not occur with fenofibrate.

Finally, in patients who are treated with drugs that are metabolized by cytochrome P450, such as certain antibiotics, immunosuppressives, and protease inhibitors, and who need LDL lowering, pravastatin is preferred because it is not metabolized by P450.

## FIBRATES

Fibrates bind to and activate peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor expressed in hepatocytes, skeletal muscle, macrophages, and the heart. (PPARs are also receptors for thiazolidinediones, as discussed in Chapter 28, Pharmacology of the Endocrine Pancreas.) After ligand binding, PPARs heterodimerize with the retinoid X receptor (RXR). This heterodimer binds to and activates peroxisome proliferator response elements (PPREs) present in the promoter region of specific genes. Activation of PPREs results in increased transcription of the target genes, and, hence, increased protein expression.

*Activation of PPARα by fibrates results in numerous changes in lipid metabolism that act to decrease plasma triglyceride levels and increase plasma HDL* (Fig. 22-8). The decrease in plasma triglyceride levels is caused by increased muscle cell expression of lipoprotein lipase, decreased hepatic expression of apolipoprotein CIII, and increased hepatic oxidation of fatty acids. The increased muscle expression of lipoprotein lipase results in increased uptake of triglyceride-rich lipoproteins, with a resultant decrease in plasma triglyceride levels. The decreased hepatic production of apolipoprotein CIII may potentiate the increased lipoprotein lipase activity, as apoCIII normally functions to inhibit interaction of triglyceride-rich lipoproteins with their receptors.

The ability of fibrates to raise plasma HDL levels is due to increased HDL production and increased reverse cholesterol transport. PPARα increases hepatocyte production of apolipoproteins AI and AII, the major apolipoproteins present in HDL. This contributes directly to increased plasma HDL. Increases in reverse cholesterol transport result from macrophage up-regulation of SR-B1 and ABCA1. Increased SR-B1 expression increases HDL binding to macrophages, and increased ABCA1 facilitates increased cellular cholesterol efflux from macrophages to HDL. Hepatocytes also increase expression of SR-B1 in response to PPARα, providing a pathway for increased transfer of cholesterol from HDL to hepatocytes, with subsequent cholesterol excretion into the bile ducts as free cholesterol or bile acids.

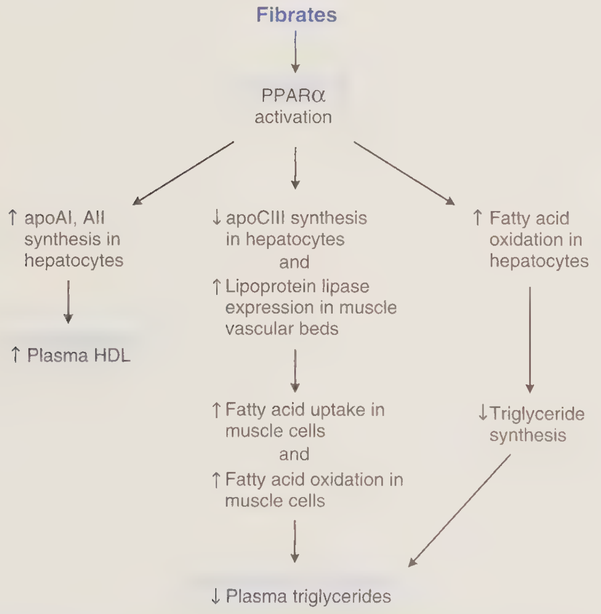

Fibrates
PPARα activation
↑ apoAI, AII synthesis in hepatocytes
↑ Plasma HDL
↓ apoCIII synthesis in hepatocytes
and
↑ Lipoprotein lipase expression in muscle vascular beds
↑ Fatty acid uptake in muscle cells
and
↑ Fatty acid oxidation in muscle cells
↓ Plasma triglycerides
↑ Fatty acid oxidation in hepatocytes
↓Triglyceride synthesis

**Figure 22-8. Effects of Fibrates on Lipid Metabolism.** Fibrates have several effects on lipid metabolism, all of which are thought to result from PPARα-mediated changes in gene transcription. PPARα activation by fibrates increases hepatic synthesis of apoAI and apoAII; this increased apolipoprotein expression increases plasma HDL. PPARα activation also decreases hepatic synthesis of apoCIII, which normally inhibits lipoprotein lipase activity. The decreased apoCIII, combined with increased lipoprotein lipase expression in muscle vascular beds, leads to increased fatty acid uptake in muscle cells and increased fatty acid oxidation. PPARα also increases fatty acid oxidation in hepatocytes. The end result of these metabolic alterations is a decrease in plasma triglyceride levels and an increase in plasma HDL levels. LDL levels also decrease modestly because of a decrease in hepatic fatty acid and triglyceride synthesis (not shown).

Fibrates also lower LDL levels modestly, although to a smaller extent than statins. The lower LDL levels result from a PPARα-mediated shift in hepatocyte metabolism toward fatty acid oxidation. PPARα increases the expression of numerous enzymes involved in fatty acid transport and oxidation; the resultant increase in fatty acid catabolism leads to decreased triglyceride synthesis and, therefore, decreased VLDL production. Because most LDL is derived from VLDL, the decreased VLDL synthesis results in decreased plasma LDL levels. Many of these effects of PPARα on lipid metabolism are still being elucidated; more detailed understanding of these effects may lead to development of more selective PPARα agonists in the future.

The fibrates available in the U.S. are **gemfibrozil** and **fenofibrate.** Two other fibrates, bezafibrate and ciprofibrate, are available in Europe, but are not approved for use in the U.S. Fibrates are indicated for treatment of hypertriglyceridemia and hypertriglyceridemia with low HDL. In addition, fibrates are the therapy of choice for patients with the type III dysbetalipoproteinemia (increased plasma levels of triglycerides and lipoprotein remnants), which results from homozygous inheritance of the apoE2 allele. The mechanism of response to fibrates in this disease is unknown, but may be related to decreased VLDL production, and, therefore, less remnant formation. Because of their greater efficacy, statins are preferred over fibrates for treatment of increased LDL levels. However, fibrates can be used together with statins in cases of combined hyperlipidemia or when HDL is decreased.

Fibrates are associated with gastrointestinal discomfort as the most common side effect. Rare side effects include myopathy and/or arrhythmias. Increases in liver transaminases occur in about 5% of patients. Gastrointestinal disturbances and myopathy are less frequent with fenofibrate than gemfibrozil. Fibrates displace warfarin from albumin binding sites, resulting in increased free warfarin concentrations; the dose of warfarin administered should, therefore, be reduced by 30% when co-administered with a fibrate. Gallstone formation has been documented as a result of increased biliary cholesterol excretion. Fibrate metabolism in relation to co-administration with a statin is described above (see p. 369). Fenofibrate increases the clearance of cyclosporine; if plasma cyclosporine levels are not monitored, patients taking cyclosporine and fenofibrate may undergo acute organ transplant rejection.

## NIACIN

Niacin (nicotinic acid, vitamin $B_3$) is a water-soluble vitamin that, at physiologic concentrations, is used in the synthesis of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), both of which are important cofactors in intermediary metabolism.

The pharmacologic applications of niacin require large (1,500–3,000 mg/day) doses, and are independent of the conversion of nicotinic acid to NAD or NADP. Niacin improves virtually every lipid parameter, resulting in decreased LDL, decreased plasma triglycerides, and increased HDL. It is also the only agent that significantly decreases Lp(a) levels. The molecular targets for niacin's actions are unknown, but several metabolic changes have been observed (Fig. 22-9). Niacin decreases adipose tissue **hormone-sensitive lipase** activity. Decreased hormone-sensitive lipase reduces peripheral tissue triglyceride catabolism, and, therefore, decreases the flux of free fatty acids to the liver. Decreased fatty acids in the liver decreases the rate of hepatic triglyceride synthesis and VLDL production. Because LDL is derived from VLDL, the decreased VLDL production ultimately decreases plasma LDL. Niacin also increases the half-life of apoAI, the major apolipoprotein present in HDL. The increased plasma apoAI increases plasma HDL concentrations and augments reverse cholesterol transport.

Pharmacologic doses of niacin are available as oral agents administered daily. The major adverse effects of niacin are **cutaneous flushing** and **pruritus** (itching). The flushing is mediated by elevated prostaglandin levels and can be prevented by pretreatment with aspirin or another NSAID. These side effects also undergo tachyphylaxis and usually disappear after several weeks of use; however, missing a single day of niacin can result in resumption of side effects. Time-release formulations of niacin are also available; this formulation decreases cutaneous flushing.

Three other important effects of niacin are hyperuricemia, impaired insulin sensitivity, and myopathy. Hyperuricemia occurs in approximately 20% of patients, and may precipitate gout (see Chapter 46, Integrative Inflammation Pharmacology: Gout). Impaired insulin sensitivity may precipitate diabetes in patients at risk; niacin should be used with caution in diabetic patients. Niacin also may rarely cause myopathy. Concurrent administration of niacin with statins slightly increases the risk of myopathy.

Niacin is indicated for patients with familial combined hyperlipidemia (elevation of both triglycerides and cholesterol), usually in combination with a statin. Because niacin is the most effective agent available for raising HDL, it may also be the drug of choice for patients with elevated LDL and decreased HDL, although the mortality benefits, if any, of this combination are still being investigated.

## ■ *Conclusion and Future Directions*

Lipid metabolism relies on lipoproteins for transport of hydrophobic lipids between tissues. The physiology of lipid metabolism can be divided into exogenous and endogenous pathways. Exogenous metabolism consists of dietary absorption of fatty acids and cholesterol from the small intestine. These lipids are esterified and packaged as chylomicrons, which then circulate in the plasma and serve as a source of free fatty acids for peripheral tissues. Endogenous lipid metabolism begins with hepatic synthesis of VLDL, a relatively triglyceride-rich lipoprotein particle. Hydrolysis of triglycerides from VLDL results in formation of IDL and eventually LDL. LDL is the major circulating source of free cholesterol. LDL may become oxidized in peripheral tissues and then taken up by macrophages; this is thought to be an initiating event in atherogenesis. HDL distributes cholesterol between tissue sources; its activity in reverse cholesterol transport may decrease or slow atherosclerosis.

Disorders of lipid metabolism include numerous monogenic diseases that disrupt pathways of cholesterol transport, as well as currently undefined polygenic risk factors; the confluence of a Western diet, obesity, and lack of exercise superimposed on these genetic risk factors predispose to increased LDL levels, increased triglycerides, reduced HDL, and increased risk of cardiovascular mortality. Five classes of pharmacologic agents are currently available to treat lipid disorders. Bile acid sequestrants interrupt enterohepatic bile acid circulation; inhibitors of cholesterol absorption limit uptake of exogenous cholesterol; and statins inhibit HMG CoA reductase, the rate-limiting step in cholesterol synthesis. These three drug classes cause increased hepatocyte LDL-R expression and, therefore, decrease plasma LDL. Fibrates are agonists of PPARα, a transcription factor that increases expression of lipoprotein lipase and apolipoprotein AI, as well

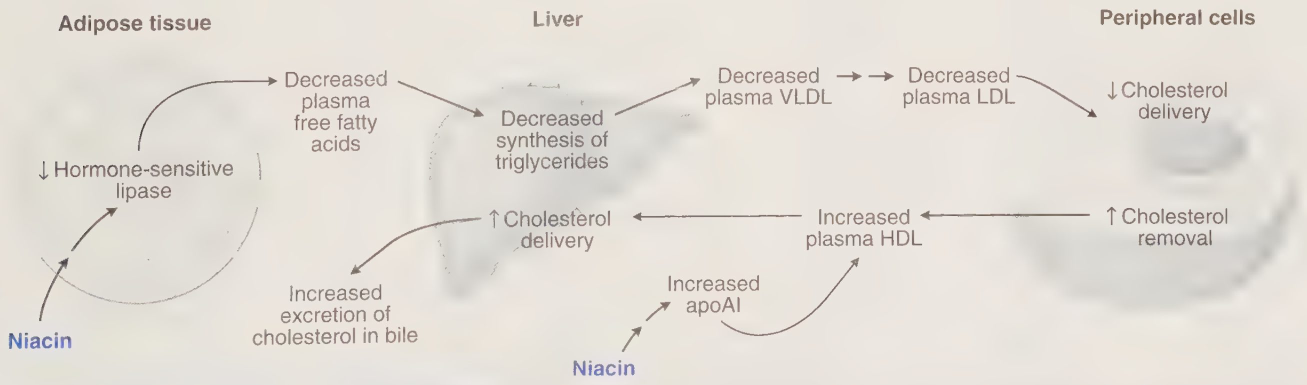


**Figure 22-9. Effects of Niacin on Lipid Metabolism.** Niacin lowers triglyceride and LDL levels while increasing HDL. The molecular mechanism of niacin action is unknown, but niacin has been shown to decrease hormone-sensitive lipase activity in adipose tissue, leading to decreased free fatty acid flux to the liver. This decreased free fatty acid flux results in decreased hepatic triglyceride synthesis and decreased VLDL synthesis. Because LDL is derived from VLDL, the decreased VLDL synthesis decreases plasma concentrations of LDL. Niacin also increases the half-life of apoAI, an important apolipoprotein in HDL. The increased apoAI levels directly increase levels of plasma HDL, and may also augment reverse cholesterol transport, delivery of cholesterol from HDL to the liver, and excretion of cholesterol in the bile.

as a number of other proteins involved in lipid metabolism; these effects combine to decrease plasma triglycerides and increase plasma HDL. High-dose niacin therapy also improves all lipid parameters, but the molecular mechanism of its action is unknown.

The efficacy of statins in reducing LDL has been an important advance in reducing cardiovascular disease mortality. Future drug trials will examine possible benefits for cardiovascular disease from raising HDL and lowering triglyceride levels, and from increasing the statin dose from starting to maximum doses. Pharmacologic therapies for new biochemical targets are also under development. These include ACAT, MTP, and CETP. ACAT inhibition may reduce cholesterol ester in atherosclerosis. Inhibition of MTP, a protein required for hepatic VLDL synthesis, reduces VLDL secretion. Modification of CETP activity alters levels of reverse cholesterol transport and, therefore, raises plasma HDL and lowers LDL.

## *Suggested Readings*

Adult Treatment Panel III. 2001. Executive summary of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. JAMA 2001;285:2486–2497. (*Clinical guidelines for cholesterol lowering therapy.*)

Kwiterovich PO. The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: A current review. Am J Cardiol 2000;86:5L–10L. (*Summarizes current understanding of lipoprotein metabolism.*)

## Drug Summary Table Pharmacology of Lipid Metabolism

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF BILE ACID ABSORPTION**<br>*Mechanism—Bind to bile acids, preventing enterohepatic circulation → LDL decreases 8%–24%, HDL increases 5%* | | | | |
| **Cholestyramine**<br>**Colestipol** | Hypercholesterolemia–second line agent | ↑ Triglyceride levels<br>Bloating<br>Dyspepsia | Hypertriglyceridemia<br>Inhibit digoxin, warfarin, and fat soluble vitamin absorption | Lower LDL levels dose-dependently, increase HDL modestly. Side effects limit the acceptable dose. |
| **INHIBITORS OF CHOLESTEROL ABSORPTION**<br>*Mechanism—Unknown* | | | | |
| **Colesevelam**<br>**Ezetimibe** | Hypercholesterolemia–second line agent | Well tolerated | None | Modest LDL reduction. No effect on HDL or TG. Colesevelam is not absorbed. Ezetimibe is absorbed and circulates systemically and enterohepatically. |
| **INHIBITORS OF CHOLESTEROL SYNTHESIS**<br>*Mechanism—Inhibit HMG CoA reductase → LDL decreases 25% to 55%, HDL increases 5%, TG decreases 10%–25%* | | | | |
| **Lovastatin**<br>**Pravastatin**<br>**Simvastatin**<br>**Fluvastatin**<br>**Atorvastatin**<br>**Rosuvastatin** | Hypercholesterolemia<br>Primary and secondary prevention<br>Diabetes | Myopathy (rare)<br>Rhabdomyolysis (rare)<br>Hepatotoxicity (rare) | Pregnancy<br>Liver disease<br>Niacin or gemfibrozil + statin ↑ myopathy risk<br>Inhibitors of CYP3A4 increase risk of myopathy | Drug of choice for lowering LDL<br>Rosuvastatin most potent<br>Pravastatin and fluvastatin NOT metabolized by CYP3A4 → less risk of drug interactions. Adverse effects of simva and atorva mainly at the highest dose, and uncommon. Minimal drug interaction with fenofibrate compared to gemfibrozil. |
| **FIBRATES**<br>*Mechanism—Agonists of PPARα → TG decreases 35% to 70%, HDL increases 5%–15%* | | | | |
| **Gemfibrozil**<br>**Fenofibrate** | Hypertriglyceridemia<br>Low HDL<br>Type III dysbetalipoproteinemia | GI discomfort<br>Myopathy<br>Arrhythmias<br>Gallstones | ↑ Warfarin levels<br>↑ Myopathy risk with statins<br>↓ Cyclosporine levels (fenofibrate) | Drug of choice for hypertriglyceridemia<br>Bezafibrate and ciprofibrate also available in Europe<br>Myopathy and GI side effects less with fenofibrate than gemfibrozil |
| **NIACIN**<br>*Mechanism—Reduce free fatty acid release from adipose tissue; increased plasma residence time for apoA1 → HDL increases 10% to 30%, LDL decreases 10%–20%, TG decreases 10%–20%* | | | | |
| **Niacin (high dose)** | Isolated low HDL<br>Low HDL with mildly elevated LDL or TG<br>Familial combined hyperlipidemia | Cutaneous flushing (severe and common)<br>Hyperuricemia and gout<br>↓ Insulin sensitivity<br>Hepatotoxicity<br>GI bleeding | ↑ Risk myopathy with statin (minimal)<br>Peptic ulcer disease<br>Gout | Flushing limits acceptability and dose. Flushing mainly occurs during first few weeks of use, and is preventable by aspirin.<br>Hepatotoxicity is dose-dependent<br>Sustained-release formulation has fewer side effects |

# 23

# Integrative Cardiovascular Pharmacology: Hypertension, Ischemic Heart Disease, and Congestive Heart Failure

April Wang Armstrong, Christopher W. Myers, Deborah C. Yeh, and Thomas P. Rocco

**Introduction**
**Case, Part I: Hypertension**
**Pathophysiology of Hypertension**
  Cardiac Function
  Vascular Function
  Renal Function
  Neuroendocrine Function
**Clinical Management of Hypertension**
  Reduction of Intravascular Volume
    *Diuretics*
  Down-Regulation of Sympathetic Tone
    *β-Adrenoceptor Antagonists*
    *α-Adrenoceptor Antagonists*
    *Central Sympatholytics*
  Modulation of Vascular Smooth Muscle Tone
    *$Ca^{2+}$ Channel Blockers*
    *$K^+$ Channel Openers*
  Neurohormonal Modulation
    *Angiotensin-Converting Enzyme Inhibitors*
    *$AT_1$ Antagonists (Angiotensin Receptor Blockers)*
  Monotherapy and Stepped Care
  Possible Demographic Factors
  Hypertensive Crisis
**Case, Part II: Ischemic Heart Disease**
**Pathophysiology of Ischemic Heart Disease**
  Chronic Coronary Artery Disease
    *Coronary Flow Reduction*
    *Endothelial Cell Dysfunction*
  Acute Coronary Syndromes
**Clinical Management of Ischemic Heart Disease**
  Chronic Coronary Artery Disease
    *β-Adrenoceptor Antagonists*
    *$Ca^{2+}$ Channel Blockers*
    *Nitrates*
    *Aspirin*
    *Lipid-Lowering Agents*
  Unstable Angina
    *Antianginal Drugs*
    *Heparin and Aspirin*
    *Glycoprotein IIb–IIIa Antagonists*
  Acute Myocardial Infarction
    *Streptokinase*
    *Anisoylated Plasminogen Streptokinase Activator Complex (APSAC)*
    *Recombinant Tissue-Type Plasminogen Activator (t-PA)*
    *Postmyocardial Infarction Management*
**Case, Part III: Congestive Heart Failure**
**Pathophysiology of Congestive Heart Failure**
  Etiologies of Contractile Dysfunction
  Cardiac Compensation
    *Frank–Starling Mechanism*
    *Cardiac Remodeling and Hypertrophy*
    *Neurohumoral Activation*
**Clinical Management of Congestive Heart Failure**
  Preload Reduction
    *Diuretics*
    *Spironolactone*
    *Venodilators*
  Afterload Reduction
    *ACE Inhibitors*
    *β-Adrenoceptor Antagonists*
    *Vasodilators*
  Inotropic Agents
    *Cardiac Glycosides*
    *Sympathomimetic Amines*
    *Phosphodiesterase Inhibitors*
  Combination Therapy
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

In Chapters 17–22, the pharmacology of the cardiovascular system is considered in the context of individual physiologic systems. For this reason, diuretics are discussed in the context of volume regulation, while $Ca^{2+}$ channel blockers are discussed in the context of vascular tone. In clinical practice, however, management of cardiovascular diseases often involves significant overlaps among these pharmacologic classes. Hypertension, for example, is commonly treated with both diuretics and $Ca^{2+}$ channel blockers. This chapter presents three common cardiovascular problems—hypertension, ischemic heart disease, and congestive heart failure—in the context of a single clinical case. For each disease, the relevant pathophysiology is discussed, followed by the pharmacologic strategies that derive from the pathophysiology. An understanding of the disease pathophysiology emphasizes the rationale for each pharmacologic intervention, as well as the potential for side effects. Often, pharmacologic agents are used in combination for these diseases, with the potential for serious drug–drug interactions. All of the pharmacologic agents in this chapter are discussed in some detail in the previous six chapters; the goal of this chapter is to integrate the pathophysiologic and pharmacologic mechanisms into an understanding of modern cardiovascular disease management.

### Case, Part I: Hypertension

Thomas Nandrunson, a 45-year-old manager at a telecommunications company, presents to the cardiology clinic for evaluation of exertional shortness of breath. Mr. Nandrunson had always been zealous in maintaining aerobic fitness, but about 6 months before his cardiology clinic visit, he began to note severe breathlessness as he approached the completion of his daily run, which concludes with a long but gentle uphill climb. During the intervening 6 months, the patient reports a progression in his symptoms to the point that, now, he rarely completes the first half of his daily run without resting. He denies chest discomfort at rest or with exercise. His family history is notable for hypertension and premature atherosclerosis. Mr. Nandrunson has never used tobacco products.

On examination, the patient is hypertensive (blood pressure, 160/102 mm Hg), and a prominent presystolic S4 is heard at the point of maximal impulse on the cardiac exam. The exam is otherwise unremarkable. The chest x-ray is reported as normal. The ECG reveals normal sinus rhythm and shows voltage criteria for left ventricular hypertrophy. Mr. Nandrunson is referred for noninvasive cardiac testing. He reaches a peak heart rate of 170 beats/minute during exercise tolerance testing on a treadmill, and has to terminate the test because of severe dyspnea after achieving only 7 METS. (METS are metabolic equivalents, a measure of energy consumption; a value of 7 METS is below normal for the patient's age.) A two-dimensional echocardiogram reveals concentric-pattern left ventricular hypertrophy, an enlarged left atrium, normal aortic and mitral valves, and abnormal left ventricular diastolic filling with a significant increase in the extent of filling during late diastole and atrial systole.

### QUESTIONS

1. Given the severity of hypertension in this case, Mr. Nandrunson will likely require at least two drugs to achieve adequate control of his blood pressure. What are the current recommendations for multidrug therapy of hypertension?
2. Thiazide diuretics have been used for many years as "first-line" therapy in patients with hypertension. Why should diuretics be used with caution in this patient?

## PATHOPHYSIOLOGY OF HYPERTENSION

Hypertension is a widely prevalent disease and a major risk factor for cardiovascular sequelae including stroke, coronary artery disease, peripheral vascular disease, congestive heart failure, and acute and chronic renal failure. Although factors that predispose to the development of hypertension have been defined, blood pressures vary widely in the general population and in the individual patient. Furthermore, the risk of target-organ complications related to hypertension bears an imperfect relationship to the magnitude of blood pressure elevation. These uncertainties, and the evolving nature of the available clinical evidence, cause periodic revisions in the consensus criteria for diagnosis of hypertension and stratification of hypertension severity, as well as the indications for treatment and definition of treatment endpoints. For example, although elevated **diastolic** blood pressure used to be the main indication for initiating antihypertensive treatment, it has come to be appreciated that elevated **systolic** blood pressure alone **(isolated systolic hypertension)** is a sufficient indication for treatment, particularly in elderly patients. The current criteria, which are listed in Table 23-1, are taken from the most recent consensus statement.

Patients may be asymptomatic despite marked elevation of systemic blood pressure, and the end-organ consequences and derivative clinical sequelae of hypertension are typically the major causes of morbidity and mortality (hence the designation of hypertension as the "**silent killer**"). For example, Mr. Nandrunson began to exhibit symptoms only after exercising for a prolonged period of time. Nonetheless, the severity of his hypertension puts him at major risk for developing coronary artery disease and other complications. Thus, effective strategies for detection and management of hypertension are critical elements in the primary prevention of cardiovascular disease.

Fortunately, the number and spectrum of agents available to treat patients with hypertension have expanded dramatically over the past two decades. These drugs can be administered as single agents (**monotherapy**) or as part of a multidrug combination regimen. Although the clinical end points of therapy can vary somewhat from patient to patient, the principal goal of treatment is to reduce the measured blood pressure, typically to levels less than 120 mm Hg systolic and less than 80 mm Hg diastolic.

**TABLE 23-1** Classification of Hypertension for Adults According to the JNC-VII

| | SYSTOLIC BLOOD PRESSURE (mm Hg) | | DIASTOLIC BLOOD PRESSURE (mm Hg) |
|---|---|---|---|
| Normal | <120 | and | <80 |
| Prehypertension | 120–139 | or | 80–89 |
| Hypertension Stage 1 (moderate) | 140–159 | or | 90–99 |
| Hypertension Stage 2 (severe) | ≥160 | or | ≥100 |

(Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, 2003)

The principal determinants of blood pressure are discussed in Chapter 20, Pharmacology of Vascular Tone. Briefly, *blood pressure is determined by the product of heart rate, stroke volume, and systemic vascular resistance* (Fig. 23-1). Heart rate is determined largely by sympathetic activity. Stroke volume depends on cardiac preload and afterload, which in turn depend on multiple variables affecting vascular tone. Systemic vascular resistance reflects the aggregate vascular tone of the arteriolar subdivisions of the systemic circulation. With regard to the treatment of hypertension, the relevant concept is that *blood pressure can be decreased by reducing heart rate, stroke volume, and/or systemic vascular resistance.* All of these factors are interrelated, and many pharmacologic agents affect more than one determinant of blood pressure.

Hypertension is typically categorized as either **primary (essential)** or **secondary hypertension.** In 90–95% of the hypertensive population, the cause of the elevation in blood pressure is unknown. Hypertension of unknown etiology is termed primary, or essential, hypertension. In contrast, patients whose elevated blood pressure can be attributed to a defined cause are said to have secondary hypertension. Although the mechanisms involved in the pathogenesis of hypertension in the majority of patients are unknown, the evaluation and treatment of hypertension are based on the physiology of normal blood pressure regulation and the potential secondary causes of pathologic blood pressure elevation.

Essential hypertension is likely to be caused by abnormalities in cardiac function, vascular function, renal function, and/or neuroendocrine function. Each of these systems has been identified as a cause of secondary hypertension. In many cases, a more complete understanding of primary hypertension awaits the elucidation of underlying genetic predispositions and/or molecular mechanisms.

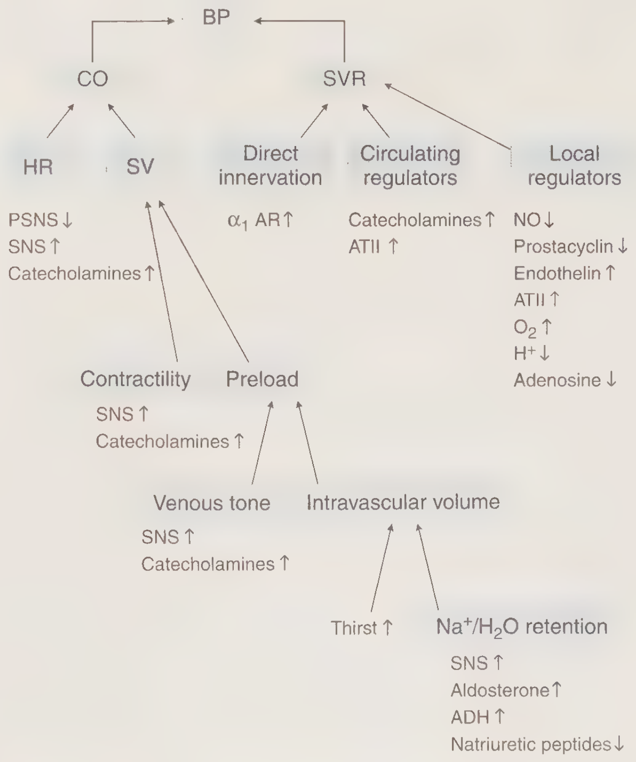


**Figure 23-1. Determinants of Systemic Blood Pressure.** Blood pressure is the product of cardiac output (CO) and systemic vascular resistance (SVR), and CO is the product of heart rate (HR) and stroke volume (SV). These determinants are altered by a number of homeostatic mechanisms. Heart rate is increased by the sympathetic nervous system (SNS) and catecholamines, and decreased by the parasympathetic nervous system (PSNS). Stroke volume is increased by contractility and preload, and decreased by afterload (not shown); all of these determinants are important parameters for cardiac function. Preload is altered by changes in venous tone and intravascular volume. The SNS and hormones, including aldosterone, antidiuretic hormone (ADH), and natriuretic peptides, are the major factors affecting intravascular volume. Systemic vascular resistance is a function of direct innervation, circulating regulators, and local regulators. Direct innervation comprises $\alpha_1$-adrenergic receptors ($\alpha_1$AR), which increase SVR. Circulating regulators include catecholamines and angiotensin II (AT II), both of which increase SVR. A number of local regulators alter SVR. These include endothelial-derived signaling molecules such as nitric oxide (NO), prostacyclin, endothelin, and AT II; and local metabolic regulators such as $O_2$, $H^+$, and adenosine. SVR is the major component of afterload, which is inversely related to stroke volume. The combination of a direct effect of SVR on blood pressure and an inverse effect of afterload on stroke volume illustrates the complexity of the system. ↑ indicates a stimulatory effect; ↓ indicates an inhibitory effect on the boxed variable.

## CARDIAC FUNCTION

One potential mechanism for persistent blood pressure elevation is a primary elevation in cardiac output (**pump-based hypertension**). A "hyperkinetic" circulation can result from excessive sympathoadrenal activity and/or increased sensitivity of the heart to basal levels of neurohumoral regulators. The hemodynamic pattern of pump-based hypertension (i.e., increased cardiac output (CO) with normal systemic vascular resistance (SVR)) is most often seen in younger patients with essential hypertension; this pattern can evolve over time into a hemodynamic profile in which the "locus" of disease appears to shift to the peripheral vasculature (see below).

## VASCULAR FUNCTION

A second mechanism for pathologic blood pressure elevation is classified as **vascular resistance-based hypertension** (normal CO, increased SVR). This hemodynamic profile is characteristic of the elderly hypertensive patient. It is postulated that, in such individuals, the blood vessels are abnormally responsive to sympathetic outflow, circulating factors, or local regulators of vascular tone. It has also been suggested that endothelial damage or dysfunction can disrupt the normal equilibrium between locally produced vasodilatory (e.g., nitric oxide) and vasoconstrictive (e.g., endothelin) factors. Finally, it seems that ion channel defects in vascular smooth muscle can cause abnormal elevations in basal vasomotor tone, resulting in increased systemic vascular resistance.

## RENAL FUNCTION

Excessive $Na^+$ and $H_2O$ retention by the kidney is responsible for **volume-based hypertension.** *Renal parenchymal disease* can result in abnormal elevation of intravascular volume, likely caused by glomerular injury with reduction of functional nephron mass and/or excessive secretion of renin. Alternatively, ion channel mutations can impair normal $Na^+$ excretion. *Renovascular disease* (e.g., renal artery stenosis caused by atherosclerotic plaques, fibromuscular dysplasia, emboli, vasculitis, or external compression) can result in decreased renal blood flow. In response to this decrease in perfusion pressure, juxtaglomerular cells increase the secretion of renin, which in turn leads to increased production of angiotensin II and aldosterone. The latter mediators increase both vasomotor tone and $Na^+/H_2O$ retention, leading to a hemodynamic profile in which both CO and SVR are elevated.

## NEUROENDOCRINE FUNCTION

Dysfunction of the neuroendocrine system—including abnormal central regulation of basal sympathetic tone, atypical stress responses, and abnormal responses to signals from baroreceptors and intravascular volume receptors—can alter cardiac, vascular, and/or renal function, so that the systemic blood pressure is increased. Examples of endocrine abnormalities associated with systemic hypertension include excessive secretion of catecholamines (pheochromocytoma), excessive secretion of aldosterone by the adrenal cortex (primary aldosteronism), and excessive production of thyroid hormones (hyperthyroidism).

## CLINICAL MANAGEMENT OF HYPERTENSION

Hypertension presents a complex challenge, because blood pressure elevation is often without symptoms for many years during which end-organ damage accumulates. As a result, the "treatment" of hypertension obligates strategies to identify both asymptomatic patients and patients at high risk for the adverse end-organ effects of the disease. In addition, long-term treatment of the hypertensive patient requires the use of drug regimens that are individualized for optimal compliance and efficacy, incorporating considerations related to both safety profile and cost.

Given the potential for adverse effects in the long-term pharmacologic treatment of hypertension, the role of primary and secondary prevention through lifestyle modifications is clear. Lifestyle modifications that have been associated with favorable results in hypertensive patients include weight loss, increased physical activity, smoking cessation, and a low-fat, low-sodium diet. Reduction or elimination of exogenous agents that can induce hypertension—such as ethanol, oral contraceptives, glucocorticoids, and stimulant drugs—has also demonstrated clinical benefit. If nonpharmacologic therapies alone do not achieve a sufficient reductions in blood pressure, such therapies can, nonetheless, contribute to successful blood pressure reduction and should be continued as adjuncts to pharmacologic treatment.

The armamentarium of drugs used to treat systemic hypertension is extensive, and there are many molecular mechanisms by which these agents exert their antihypertensive effects. It is important to remember, however, that all of these mechanisms must ultimately reduce cardiac output and/or systemic vascular resistance to achieve an antihypertensive effect. Strategies currently used to treat hypertension (Table 23-2 and Fig. 23-2) include reduction of intravascular volume (diuretics), down-regulation of sympathetic tone ($\beta$-blockers, $\alpha_1$-blockers, central sympatholytics), modulation of vascular smooth muscle tone (calcium channel blockers and $K^+$ channel openers), and inhibition of the neurohumoral regulators of the circulation (ACE inhibitors, $AT_1$ antagonists [angiotensin II receptor blockers]). The reduction in blood pressure caused by these agents is sensed by baroreceptors and renal juxtaglomerular cells, which can activate counter-regulatory responses that attenuate the magnitude of blood pressure reduction. These compensatory responses can be substantial, and can obligate dose adjustments and/or the use of more than one agent to achieve long-term blood pressure control (Fig. 23-3).

## REDUCTION OF INTRAVASCULAR VOLUME

Reduction of intravascular volume is a long-standing therapy for hypertension. As discussed in Chapter 19, diuretics decrease intravascular volume by increasing renal excretion of $Na^+$ and $H_2O$; these agents also have a direct vasodilator effect that remains poorly understood. Both the volume reduc-

**TABLE 23-2** Major Classes of Antihypertensive Agents

| DIURETICS | SYMPATHOLYTICS | VASODILATORS | RENIN–ANGIOTENSIN SYSTEM BLOCKERS |
|---|---|---|---|
| Thiazide diuretics | CNS sympathetic outflow blockers | Calcium channel blockers | ACE inhibitors |
| Loop diuretics | Ganglionic blockers | Minoxidil | $AT_1$ antagonists |
| $K^+$-sparing diuretics | Postganglionic adrenergic nerve terminal blockers | Hydralazine | |
| | $\alpha_1$-Adrenergic receptor blockers | Sodium nitroprusside | |
| | $\beta_1$-Adrenergic receptor blockers | | |
| | Mixed α-adrenergic receptor/β-adrenergic receptor blockers | | |

tion and vasodilation effected by diuretics seem to contribute to their clinical efficacy.

## Diuretics

**Thiazide diuretics** (e.g., **hydrochlorothiazide**) are the mostly commonly prescribed family of natriuretic drugs used

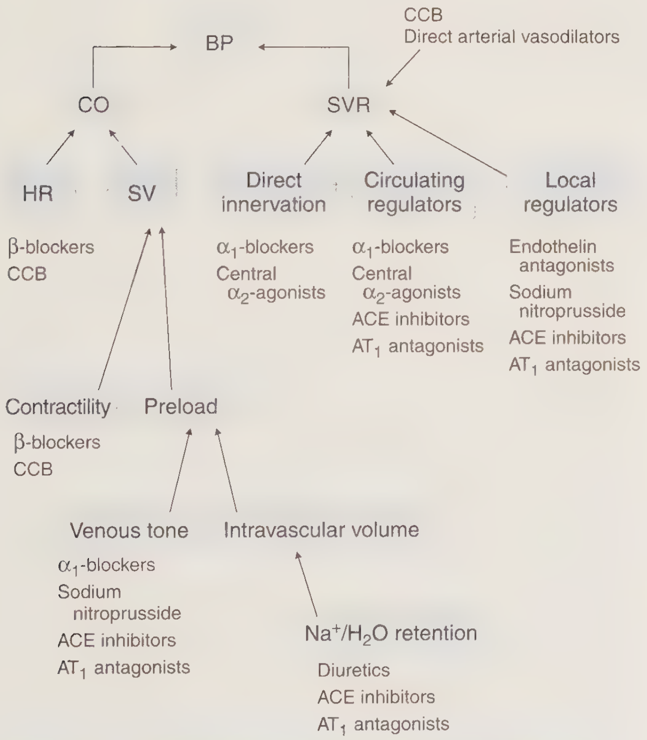

BP
CO
SVR
CCB
Direct arterial vasodilators
HR
SV
Direct innervation
Circulating regulators
Local regulators
β-blockers
CCB
$\alpha_1$-blockers
Central $\alpha_2$-agonists
$\alpha_1$-blockers
Central $\alpha_2$-agonists
ACE inhibitors
$AT_1$ antagonists
Endothelin antagonists
Sodium nitroprusside
ACE inhibitors
$AT_1$ antagonists
Contractility
Preload
β-blockers
CCB
Venous tone
Intravascular volume
$\alpha_1$-blockers
Sodium nitroprusside
ACE inhibitors
$AT_1$ antagonists
$Na^+/H_2O$ retention
Diuretics
ACE inhibitors
$AT_1$ antagonists

**Figure 23-2. Pharmacologic Effects of Commonly Used Anti-Hypertensive Agents.** Antihypertensive agents modulate blood pressure by interfering with the determinants of blood pressure. Many of these antihypertensive drugs have multiple actions. For example, renin–angiotensin system blockers, such as ACE inhibitors and $AT_1$ antagonists, alter the levels of local regulators and circulating regulators, and affect renal $Na^+$ retention and venous tone. BP, blood pressure; CO, cardiac output; SVR, systemic vascular resistance; HR, heart rate; SV, stroke volume; CCB, $Ca^{2+}$ channel blockers; ACE, angiotensin converting enzyme.

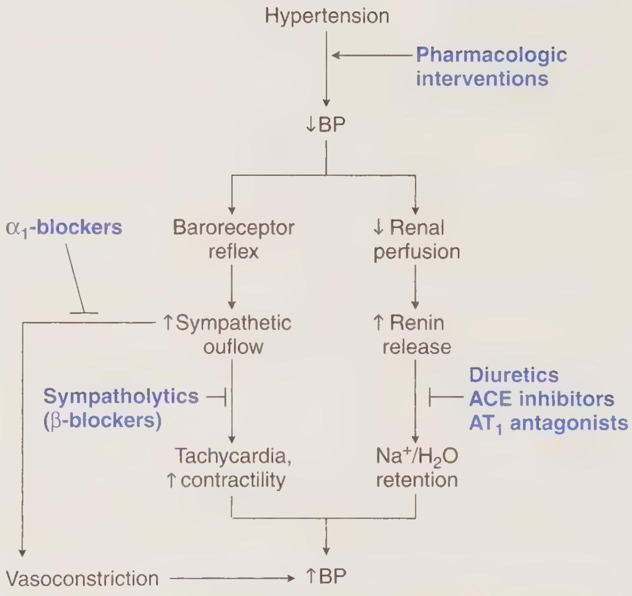

Hypertension
Pharmacologic interventions
↓BP
$\alpha_1$-blockers
Baroreceptor reflex
↓ Renal perfusion
↑Sympathetic ouflow
↑ Renin release
Sympatholytics (β-blockers)
Diuretics
ACE inhibitors
$AT_1$ antagonists
Tachycardia, ↑contractility
$Na^+/H_2O$ retention
Vasoconstriction
↑BP

**Figure 23-3. Compensatory Homeostatic Responses to Anti-Hypertensive Treatment.** When blood pressure is lowered by pharmacologic interventions, homeostatic responses are activated to increase blood pressure. These homeostatic responses can be divided broadly into baroreceptor reflexes and renal perfusion reflexes. Baroreceptor reflexes originating in the aortic arch and carotid sinus increase sympathetic outflow, leading to tachycardia, increased contractility, and vasoconstriction; these effects all increase blood pressure. Sympatholytics, such as β-blockers, blunt the tachycardia and contractility responses by interrupting the sympathetic signaling system. $\alpha_1$-Blockers inhibit vasoconstriction, but have minimal effects on tachycardia or contractility. Decreased renal perfusion causes increased release of renin from juxtaglomerular cells of the kidney. Renin then cleaves angiotensinogen to angiotensin I, which, in turn, is activated to the potent vasoconstrictor angiotensin II (not shown). Angiotensin II increases adrenal secretion of aldosterone, which acts on principal cells of the collecting duct to increase $Na^+$ (and, therefore, water) reabsorption. The increased $Na^+$ reabsorption increases intravascular volume, and, therefore, results in increased blood pressure. Diuretics interrupt this homeostatic response by decreasing $Na^+$ reabsorption from the nephron, while angiotensin-converting enzyme (ACE) inhibitors or $AT_1$ antagonists interrupt the formation or target-organ signaling of angiotensin II, respectively.

in the treatment of hypertension. The pharmacokinetic and pharmacodynamic characteristics of the thiazides—including oral availability, long duration of action, and moderate intensity of diuresis—make them especially useful agents in the treatment of chronic hypertension. Their initial antihypertensive effect seems to be mediated by decreasing intravascular volume. Therefore, the thiazides are particularly effective in patients with volume-based hypertension. The maximal antihypertensive effect of the thiazides is frequently achieved at doses lower than those needed to achieve a maximal diuretic effect, suggesting that their vasodilator effect (which lowers systemic vascular resistance) also contributes to their efficacy as antihypertensives.

In the initial Joint National Commission "Stepped-Care" algorithm for the treatment of hypertension, thiazide diuretics were recommended as the first-line "agents of choice" for the majority of patients. However, retrospective subgroup analyses raised concerns related to increased adverse cardiovascular events in certain patient cohorts, and these concerns, together with a significant incidence of undesirable metabolic effects (e.g., hypokalemia, hypercalcemia, hyperglycemia, hyperlipidemia, and hyperuricemia), have led to the current use of alternative antihypertensive drug classes as first-line therapy. For this reason, a thiazide diuretic would probably not be the first choice of antihypertensive agent in treating Mr. Nandrunson. However, a thiazide diuretic could be appropriate at low doses in combination with other antihypertensives (see below).

**Loop diuretics** (e.g., **furosemide**) are infrequently prescribed for the treatment of mild or moderate hypertension. These agents typically have a relatively short duration of action (4–6 hr) and, despite the brisk diuresis that follows their administration, their antihypertensive efficacy is often modest. It is thought that this modest impact on systemic blood pressure is due to the activation of compensatory responses involving the neurohumoral regulators of circulatory homeostasis. There are, however, several well-recognized clinical situations in which loop diuretics are preferable to thiazides, including malignant hypertension (see below) and volume-based hypertension in patients with renal insufficiency.

**$K^+$-sparing diuretics** (e.g., **spironolactone, triamterene, amiloride**) are less efficacious than thiazide and loop diuretics, and are used primarily in combination with other diuretics to attenuate or correct drug-induced kaliuresis ($K^+$ excretion) and hypokalemia. Spironolactone is an aldosterone receptor antagonist that is especially effective in the treatment of hypertension caused by hyperaldosteronism. As noted above, hypokalemia is a common metabolic side effect of the thiazide and loop diuretics, which inhibit $Na^+$ reabsorption in the more proximal segments of the nephron and, thereby, increase delivery of $Na^+$ and water to distal segments of nephron. Increased distal $Na^+$ delivery results in a compensatory increase in $Na^+$ reabsorption in the distal tubule, which is coupled to an increase in $K^+$ excretion. Because the latter effect is mediated by aldosterone (see Chapter 19), the $K^+$-sparing diuretics attenuate this effect and, thereby, help to maintain normal serum potassium levels. It should be emphasized that both ACE inhibitors (which decrease aldosterone activity and $K^+$ excretion) and $K^+$ supplements should be decreased or eliminated in patients taking $K^+$-sparing diuretics, because life-threatening hyperkalemia has been reported in association with clinical use of the $K^+$-sparing diuretics.

## DOWN-REGULATION OF SYMPATHETIC TONE

Drugs that modulate adrenergic activity are discussed in Chapter 8; refer to that chapter for detailed descriptions of the tissue distribution of α and β receptors and the cardiovascular effects mediated by these receptors. Sympatholytic drugs treat hypertension via two major mechanisms: *reduction of systemic vascular resistance and/or reduction of cardiac output.* Clinically, these agents are broadly divided into β-adrenoceptor antagonists, α-adrenoceptor antagonists, and central sympatholytics.

### β-Adrenoceptor Antagonists

β-Adrenoceptor antagonists (e.g., **propranolol, atenolol**) are among the most commonly prescribed first-line agents for hypertension. The negative chronotropic and inotropic effects of these agents (and the reductions in heart rate, stroke volume, and cardiac output that follow) account for the initial antihypertensive effect of the β-blockers. Decreased vasomotor tone, with a consequent decrease in systemic vascular resistance, has also been reported with longer-term therapy.

The β-blocker–induced reduction in vasomotor tone may seem paradoxical, given that $\beta_2$-adrenergic receptors in the peripheral vasculature mediate vasodilation. However, blockade of $\beta_1$-adrenergic receptors in the kidney decreases the secretion of renin and, thereby, decreases the production of the potent vasoconstrictor angiotensin II. The latter effect likely predominates, even when nonselective β-receptor antagonists are administered. Central effects of the β-blockers may also contribute to their antihypertensive action. Although β-blockers effectively reduce blood pressure in hypertensive patients, these agents typically do not cause hypotension in individuals with normal blood pressure. Increased baseline sympathetic activity in hypertensive patients may in part explain the efficacy of β-blockers in lowering blood pressure in these individuals. In contrast, basal activation of β-receptors in normal subjects may be sufficiently low that receptor antagonists have little hemodynamic effect.

β-Blocker therapy has been associated with both elevation of serum triglyceride levels and reduction of high-density lipoprotein levels; the clinical significance of these metabolic effects remains unclear. Noncardiac side effects of β-blocker therapy include exacerbation of glucose intolerance (hyperglycemia), sedation, impotence, and bronchoconstriction.

**Mixed α–β antagonists** (e.g., **labetalol**) are available in both oral and parenteral formulations. Intravenous administration of labetalol causes a substantial reduction in the systemic blood pressure. Use of the intravenous dosage form is typically limited to the treatment of hypertensive emergencies, including the treatment of pheochromocytoma-associated hypertension and hypertensive crisis. Oral labetalol is used in the long-term treatment of hypertension. One potential advantage of this drug is that the decrease in blood pressure achieved by reduction of systemic vascular resistance (via blockade of $\alpha_1$-receptors) is not associated with the reflex increase in heart rate or cardiac output (because cardiac $\beta_1$-receptors are also blocked) that can occur when pure vasodilator drugs are used as monotherapy.

## α-Adrenoceptor Antagonists

$\alpha_1$-Adrenergic antagonists (e.g., **prazosin, terazosin, doxazosin**) are frequently used in the treatment of high blood pressure. $\alpha_1$-Adrenergic antagonists inhibit peripheral vasomotor tone, reducing vasoconstriction and decreasing systemic vascular resistance. The absence of adverse effects on the serum lipid profile during long-term treatment with $\alpha_1$-adrenergic antagonists is often cited as a distinctive advantage of these agents relative to other antihypertensive medications. However, the long-term benefit of this advantage, if any, remains to be determined in randomized clinical trials.

Nonselective α-adrenergic blockers (e.g., **phenoxybenzamine, phentolamine**) are not employed in the long-term treatment of hypertension, because excessive compensatory responses result from their long-term use. For example, blockade of central $\alpha_2$-adrenergic receptors disinhibits sympathetic outflow, resulting in unopposed reflex tachycardia. One clinical setting in which these agents are specifically indicated is the treatment of pheochromocytoma.

## Central Sympatholytics

Blockers of sympathetic outflow from the central nervous system include the **$\alpha_2$-adrenergic agonists methyldopa, clonidine,** and **guanabenz.** These agents decrease sympathetic outflow from the medulla, leading to decreases in heart rate, contractility, and vasomotor tone. These drugs are available in oral formulations (clonidine is also available as a transdermal patch), and were widely used in the past despite their unfavorable side effect profile. The availability of multiple alternative agents, as well as the current trend toward use of multidrug regimens at submaximal doses, have substantially reduced the clinical role of the $\alpha_2$-agonists in the treatment of hypertension.

**Ganglionic blockers** (e.g., **trimethaphan, hexamethonium**) inhibit nicotinic cholinergic activity at sympathetic ganglia. These agents are extremely effective at lowering blood pressure. However, the severe adverse effects of parasympathetic and sympathetic blockade (e.g., constipation, blurred vision, sexual dysfunction, and orthostatic hypotension) have made ganglionic blocker agents of historic interest only.

Blockers of neurotransmission at adrenergic nerve terminals (e.g., **reserpine, guanethidine**) are taken up into the terminals of postganglionic adrenergic neurons, where they enter norepinephrine synaptic vesicles and, eventually, induce degradation of the neurotransmitter (see Chapter 8). These agents reduce blood pressure by decreasing the activity of the sympathetic nervous system. Reserpine and guanethidine have little role in the contemporary treatment of hypertension, however, because of the significant adverse effects associated with their use, including severe depression (reserpine) and orthostatic hypotension and sexual dysfunction (guanethidine).

# MODULATION OF VASCULAR SMOOTH MUSCLE TONE

As discussed in Chapter 20, vascular tone is dependent on the degree of vascular smooth muscle contraction. Vasodilators reduce peripheral systemic vascular resistance by acting on arteriolar smooth muscle and/or the overlying endothelium. The major mechanisms of action of the arterial vasodilators include blockade of $Ca^{2+}$ channels and opening of metabotropic $K^+$ channels. Drugs that elaborate nitric oxide, which is also a potent vasodilator, are not commonly employed in the treatment of hypertension. Instead, these agents are more commonly prescribed in the treatment of ischemic heart disease (see below).

## $Ca^{2+}$ Channel Blockers

$Ca^{2+}$ channel blockers (e.g., **verapamil, diltiazem, nifedipine, amlodipine**) are orally bioavailable and are widely used in the long-term treatment of hypertension. These agents can act as arterial vasodilators, negative inotropes, and/or negative chronotropes. Calcium channel blockers (**CCBs**) have a variety of hemodynamic effects, reflecting the multiple sites at which calcium is involved in the electrical and mechanical events of the cardiac cycle and in vascular regulation. The dihydropyridine agents (nifedipine and amlodipine) act primarily as vasodilators. In contrast, the nondihydropyridine drugs verapamil and diltiazem act principally as negative inotropes and chronotropes, thereby decreasing myocardial contractility, heart rate, and impulse conduction. Thus, *the CCBs can decrease blood pressure through reduction of both systemic vascular resistance and cardiac output.*

The CCBs are often used in combination with other cardioactive drugs, either as components of a multidrug antihypertensive regimen or as antihypertensive treatment in patients with concomitant cardiovascular disease states for which these drugs could have an additional therapeutic benefit (e.g., ischemic heart disease). Given the distinctive pharmacodynamic effects of the different CCBs, the potential side effects of CCB therapy (including adverse interactions with other cardiovascular therapies) are agent specific. For example, the nondihydropyridine agents should be used with caution in patients who have impaired LV systolic function and/or conduction system disease because these agents can exacerbate heart failure, SA node dysfunction, and AV node conduction abnormalities. This caution is particularly relevant in patients receiving simultaneous β-blocker therapy, because both β-blockers and CCBs decrease AV nodal conduction.

## $K^+$ Channel Openers

**Minoxidil** and **hydralazine** are orally available arterial vasodilators that can be used in the long-term treatment of hypertension. Minoxidil is a metabotropic $K^+$ channel opener that hyperpolarizes vascular smooth muscle cells and, thus, attenuates the cellular response to depolarizing stimuli. Hydralazine is a less powerful vasodilator whose mechanism of action remains uncertain. Both minoxidil and hydralazine can cause compensatory retention of $Na^+$ and $H_2O$ as well as reflex tachycardia; these adverse effects are more frequent and more severe with minoxidil than with hydralazine. Concomitant use of a β-blocker and a diuretic can mitigate these compensatory side effects. The use of hydralazine is limited by the frequent occurrence of tolerance and tachyphylaxis to the drug. In addition, increases in the total daily dose of hydralazine are associated with a drug-induced lupus syndrome. Given the more favorable safety profile of the $Ca^{2+}$ channel blockers, the use of both minoxidil and hydralazine is now largely restricted to patients with severe hypertension that is refractory to other pharmacologic therapies.

## NEUROHORMONAL MODULATION

Renin–angiotensin system blockers include the **ACE inhibitors** (e.g., **captopril, enalapril, lisinopril**) and the **angiotensin receptor ($AT_1$) antagonists** (e.g., **losartan, valsartan**).

### Angiotensin-Converting Enzyme Inhibitors

**ACE inhibitors** prevent the ACE-mediated conversion of angiotensin I to angiotensin II, leading to decreased circulating levels of angiotensin II and aldosterone. By decreasing the level of the vasoconstrictor angiotensin II, ACE inhibitors decrease peripheral (systemic) vascular resistance and, thereby, decrease the impedance to LV ejection. These agents also promote natriuresis, and consequently reduce intravascular volume, by decreasing aldosterone levels. Furthermore, ACE inhibitors decrease bradykinin breakdown, and the resulting increase in circulating levels of bradykinin causes vasodilation. ACE inhibitors are effective in patients with hyper-reninemic hypertension, but these agents also reduce blood pressure in patients with low-to-normal circulating renin levels. The effectiveness of ACE inhibitors in lowering blood pressure in patients without excessive plasma renin activity may be due to potentiation of the vasodilatory effects of bradykinin, although this hypothesis remains to be proved.

Monotherapy with ACE inhibitors is as effective as monotherapy with thiazide diuretics or β-blockers in the treatment of hypertension. ACE inhibitors are attractive antihypertensive agents because, compared to thiazide diuretics and β-blockers, these drugs seem to have some additional benefits (e.g., nephroprotective properties in diabetics) and fewer adverse effects. Unlike thiazide diuretics, ACE inhibitors do not increase the risk of hypokalemia (although they may increase the risk of hyperkalemia), and unlike both β-blockers and thiazide diuretics, ACE inhibitors do not elevate serum glucose or lipid levels.

ACE inhibitors should be administered with caution in patients with intravascular volume depletion. Such patients may have reduced renal perfusion at baseline, leading to a compensatory increase in renin and angiotensin II; this increase in angiotensin II is one of the physiologic mechanisms by which GFR is maintained in the face of relative renal hypoperfusion. Administration of ACE inhibitors to such patients can disrupt this autoregulatory mechanism, leading to renal insufficiency. The same autoregulatory mechanism is the basis for the contraindication to ACE inhibitors in patients with bilateral renal artery stenosis (or unilateral stenosis in patients with a single kidney). Despite these cautionary notes, it should be emphasized that ACE inhibitors are considered the *preferred* therapy in the hypertensive diabetic patient: ACE inhibitors have been shown to delay the onset and progression of diabetic glomerular disease through favorable effects on intraglomerular pressure.

### $AT_1$ Antagonists (Angiotensin Receptor Blockers)

Angiotensin II receptor ($AT_1$) antagonists (also known as **angiotensin receptor blockers** or **ARBs**) are oral antihypertensive agents that competitively inhibit the binding of angiotensin II to its cognate $AT_1$ receptors. $AT_1$ antagonists may also reduce reactive arteriolar intimal proliferation. $AT_1$ antagonists are as effective as ACE inhibitors in lowering blood pressure, and are commonly substituted for ACE inhibitors in patients with drug-induced cough. (Note that cough, a common side effect of ACE inhibitor therapy, results from drug-induced increases in bradykinin levels; this side effect often leads to noncompliance or discontinuation of the drug. Because $AT_1$ antagonists do not affect the activity of the converting enzyme responsible for bradykinin degradation, cough is not a side effect of therapy with the ARBs.)

## MONOTHERAPY AND STEPPED CARE

**Monotherapy** (treatment with a single drug) is often sufficient to normalize blood pressure in patients with mild hypertension. To date, thiazide diuretics and β-blockers are the only drugs that have been shown to reduce mortality (in elderly hypertensive patients), and these drugs are now advocated by many as first-line therapies. However, the adverse metabolic effects of thiazide diuretics and β-blockers are a persistent concern that underlies the advocacy by some for less restrictive recommendations regarding first-line antihypertensive therapies. Alternative options for initial monotherapy include ACE inhibitors (e.g., captopril), $Ca^{2+}$ channel blockers (e.g., amlodipine), selective $\alpha_1$-blockers (e.g., prazosin), mixed α–β blockers (e.g., labetalol), and central sympatholytic agents (e.g., clonidine).

The results of large clinical trials indicate that adequate blood pressure control can be achieved for many patients with a single drug. Although monotherapy for mild and moderate hypertension may improve patient compliance and avoid the risk of potential drug interactions, it must be emphasized that side effects and drug toxicities are often related to drug dose, and that treatment efficacy may not be explicable on the basis of the degree of blood pressure reduction alone. The clinician must carefully consider the use of "synergistic" agents at lower doses, especially if blood pressure control is marginal or inadequate.

**Stepped care** in the treatment of hypertension refers to the progressive, step-by-step addition of drugs to a therapeutic regimen. Combination therapy is based on the use of agents with distinct mechanisms of action; it also emphasizes the use of submaximal doses of drugs in an attempt to minimize potential side effects and toxicities. Historically, therapies have been added to a patient's regimen in the following order, as needed: lifestyle modifications, diuretics, sympatholytics, vasodilators, and, finally, renin–angiotensin system blockers. Although early iterations of the stepped-care approach were somewhat rigid, more recent algorithms provide far greater latitude in selecting the order of addition of the various drug classes. Current treatment algorithms recognize that any given drug will likely have effects on more than one of the interrelated systems that regulate circulatory function. For example, the initial decrease in mean arterial pressure induced by short-acting arterial vasodilators, such as hydralazine or nifedipine, activates compensatory mechanisms that can result in significant tachycardia and $Na^+$ and $H_2O$ retention. The addition of a sympatholytic drug (e.g., propranolol) and/or a diuretic (e.g., hydrochlorothiazide) can mitigate these compensatory responses. Furthermore, pharmaceutical advances have allowed for novel drug formulations that can alter the kinetics of drug metabolism and elimination. For example, the problem of compensatory tachycardia in response to short-acting CCBs of the dihydropyridine class has, to a great extent, been circumvented by the development of sustained-release formulations and of alternative drugs with more favorable pharmacokinetic profiles (see Chapter 20).

## POSSIBLE DEMOGRAPHIC FACTORS

Certain classes of antihypertensive drugs have been reported to be more effective than others in special populations. Some data also suggest that distinct etiologies of hypertension may be more or less prevalent in different populations. **Elderly patients** tend to respond more favorably to diuretics and $Ca^{2+}$ channel blockers than to other antihypertensive agents. β-Blockers are more likely to cause SA or AV node dysfunction or to impair myocardial function in elderly patients; these effects are likely related to the greater prevalence of conduction system disease and LV systolic dysfunction in such patients. Elderly patients also tend to have decreased circulating levels of renin, and have been reported to be less responsive to ACE inhibitors.

It has been reported that hypertension in patients of **African descent** is more responsive to diuretics and $Ca^{2+}$ channel blockers than to β-blockers or ACE inhibitors. A notable exception is the favorable response of young African Americans to β-blocker therapy. Reports indicate that some African Americans may have lower circulating renin levels, and this could account for the observation that ACE inhibitors are less effective in these patients. Recent reports have demonstrated that the prevalence of $Na^+$-sensitivity is substantially increased in some African Americans, including both the hypertensive and the normotensive cohort. Although less well studied, there is some evidence of differential responsiveness to the various classes of antihypertensive agents in hypertensive **Asian** and **Hispanic** cohorts.

Despite these interesting demographic observations, the clinical benefit of drug selection on the basis of differential responsiveness to specific drug classes has not been evaluated systematically. For example, although elderly patients are reportedly less responsive to β-blockers, the results of the Systolic Hypertension in the Elderly Project (SHEP Trial) indicate that β-blockers and diuretics are, in fact, associated with mortality reduction, and that this favorable treatment effect is demonstrated within several years of treatment initiation. Similarly, although reports have suggested that African Americans are less responsive to ACE inhibitors and β-blockers, it would be difficult to apply these observations to the treatment of a hypertensive, diabetic African American with an elevated serum creatinine, or to advocate for the use of a thiazide diuretic in a hypertensive African American with a history of previous myocardial infarction. Finally, it should again be emphasized that the "risk" of adverse events related to hypertension cannot be explained by the degree of blood pressure elevation alone. Conversely, the full spectrum of treatment benefits cannot be explained by the degree of blood pressure reduction alone. For these reasons, the empirical observation that some antihypertensives do not lower blood pressure as effectively in some patients does not necessarily mean that these drugs will be less effective in preventing future cardiovascular disease morbidity and mortality in those patients. Clearly, more research is needed.

## HYPERTENSIVE CRISIS

The term "hypertensive crisis" refers to clinical syndromes characterized by severe (typically acute) elevations in blood pressure. This abrupt increase in blood pressure can cause acute vascular injury and derivative end-organ damage. Although most cases of severe hypertension were, at one time, designated as "hypertensive crisis" or "malignant hypertension," current practice attempts to distinguish those patients in whom the blood pressure elevation and vascular injury are acute (**hypertensive emergency**) from the patient cohort in which the temporal course of blood pressure elevation is more gradual, and the end-organ damage is chronic and slowly progressive.

A true hypertensive emergency is, therefore, a life-threatening condition in which severe and acute blood pressure elevation is associated with acute vascular injury. The vascular injury can manifest clinically as retinal hemorrhages, papilledema, encephalopathy, and acute (or acute superimposed on chronic) renal insufficiency. This syndrome is often associated with acute left ventricular failure. The pathogenesis of malignant hypertension remains unclear. However, it is likely that **fibrinoid arteriolar necrosis** contributes to the signs and symptoms of this syndrome. Fibrinoid arteriolar necrosis of particular vascular beds can result in acute vascular injury and end-organ hypoperfusion (e.g., renal failure in the kidneys, stroke in the brain). Fibrinoid arteriolar necrosis can also lead to microangiopathic hemolytic anemia.

The treatment of patients with hypertensive emergency necessitates rapid reduction of the blood pressure to prevent end-organ damage. Drug classes used for this condition include parenteral vasodilators (e.g., nitroprusside), diuretics (e.g., furosemide), and/or β-blockers (e.g., labetalol). Because of the acuity of the syndrome and the need to titrate carefully these powerful antihypertensive agents, patients are hospitalized for treatment. After the acute episode has been controlled, subsequent lowering of the blood pressure to the "normal" range of the patient is then attempted more cautiously over a longer period of time (12–24 hr), in an effort to decrease the risk of critical-organ hypoperfusion and extension of the vascular injury.

Although malignant hypertension is a life-threatening medical emergency, it is an uncommon expression of hypertensive disease that occurs in far less than 1% of hypertensive patients. More common are cases of "**hypertensive urgency,**" in which the blood pressure elevation is less acute and the existing target organ disease has been present for some time. Conditions illustrative of hypertensive urgency include a cerebrovascular accident (stroke) or myocardial infarction that is accompanied by severe blood pressure elevation, or acute left heart failure with severe hypertension and aortic dissection.

## Case, Part II: Ischemic Heart Disease

Mr. Nandrunson is treated for hypertension with a β-blocker and an ACE inhibitor. He returns for follow-up visits at 1 month and 6 months and reports that he is doing well. He has faithfully adhered to his prescribed medical regimen and has noted a definite improvement in exercise capacity. His regular blood pressure measurements have shown readings of 130–150/86–90 mm Hg. A serum lipid profile is notable for increased total cholesterol, with a moderately elevated LDL. Low-dose aspirin is added to his regimen. Treatment with a lipid-lowering agent is also advised, but Mr. Nandrunson declines, instead requesting that his profile be rechecked after a period of diet and lifestyle modifications.

An exercise tolerance test 1 year after his initial visit is notable for improved exercise capacity (10 MET workload), with blunting of the heart rate and blood pressure at peak exercise (120/minute and 190/90 mm Hg, respectively); there is no evidence of myocardial ischemia by ECG criteria. A repeat LDL cholesterol determination is within the normal range. His medications are continued and routine follow-up is established.

One week later, however, Mr. Nandrunson experiences the abrupt onset of severe retrosternal chest pressure. He is visibly diaphoretic and dyspneic. He calls 911 and is transported to the local ER, where an ECG shows sinus bradycardia and ST segment elevation in the inferior leads. Emergent cardiac catheterization is performed, confirming total occlusion of a dominant right coronary artery, and percutaneous transluminal coronary angioplasty (PTCA) with stent placement is also performed. Although the PTCA and stent placement are successful, and he remains free of chest pain and is hemodynamically stable, ECG and enzymatic (peak creatine kinase (CK), 2300 IU/L; cardiac isoform (MB) fraction, positive) changes are consistent with an evolving myocardial infarction. A repeat echocardiogram immediately before Mr. Nandrunson's discharge from the hospital shows left ventricular hypertrophy with a left ventricular ejection fraction of 40% (normal, >55%); the basal segment of the inferior wall is akinetic, with thinning of the myocardium in this akinetic region.

### QUESTIONS

1. What type of lipid lowering agent is appropriate for this patient?
2. What pharmacologic interventions are appropriate during the interval between the patient's ER evaluation and his cardiac catheterization?
3. What are the critical drug components of a post-myocardial infarction treatment regimen?

## PATHOPHYSIOLOGY OF ISCHEMIC HEART DISEASE

Ischemic heart disease (IHD), the leading cause of mortality in the United States, accounts for more than 500,000 deaths per year. Over the past decades, an improved understanding of the biology of ischemic heart disease has resulted in a spectrum of diagnostic and therapeutic advances. These advances, coupled with increased public awareness, healthier lifestyle choices, and sustained efforts to improve both primary and secondary prevention strategies, have resulted in significant mortality reduction for patients with ischemic heart disease.

With respect to pharmacotherapy, IHD can be divided into two broad categories: **chronic coronary artery disease** (CAD) and **acute coronary syndromes.** Each of these clinical presentations of IHD has a distinct pathogenesis and, as a result, the pharmacologic strategies employed to treat these entities differ in emphasis. The therapeutic goal in patients with chronic coronary artery disease is to *maintain a balance between myocardial oxygen supply and demand;* in patients with acute coronary syndromes, the goal is to *restore and/or maintain patency of the vascular lumen* (Fig. 23-4).

## CHRONIC CORONARY ARTERY DISEASE

Chronic coronary artery disease is typically associated with impaired coronary vasodilator reserve. Under conditions of hyperemic stress (i.e., stress requiring increased blood flow), an imbalance between myocardial oxygen supply and demand can occur, leading to functional cardiac abnormalities as well as clinical symptoms of CAD. The basic physiology of myocardial oxygen supply and demand is discussed in Chapter 20. Imbalances in myocardial oxygen supply and demand occur mainly as a result of coronary flow reduction and endothelial cell dysfunction.

### Coronary Flow Reduction

The coronary vasculature is comprised of two types of vessels: large, proximal epicardial vessels, and small, distal endocardial vessels. The epicardial vessels are the more frequent site of atheroma formation; in disease states, total coronary artery blood flow is limited by the extent of epicardial vessel occlu-

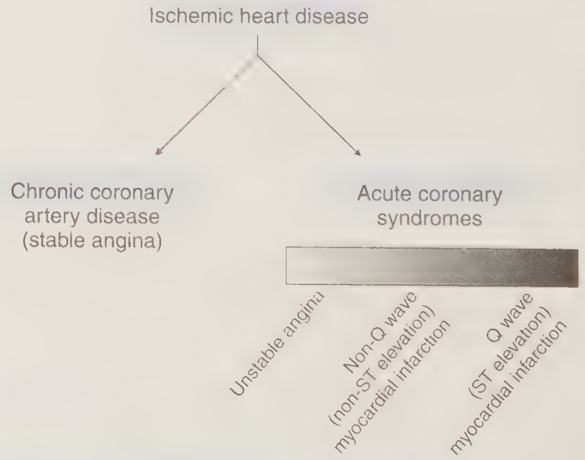

Ischemic heart disease
Chronic coronary artery disease (stable angina)
Acute coronary syndromes
Unstable angina
Non-Q wave (non-ST elevation) myocardial infarction
Q wave (ST elevation) myocardial infarction

**Figure 23-4. Classification of Ischemic Heart Disease.** Ischemic heart disease is divided into two broad categories: chronic coronary artery disease and acute coronary syndromes. Stable angina is the prototypical manifestation of chronic coronary artery disease. Acute coronary syndromes comprise a spectrum (not necessarily a linear progression) of clinical presentations, from unstable angina, to non-Q wave (non-ST elevation) myocardial infarction, to Q wave (ST elevation) myocardial infarction.

sion. In comparison, endocardial vessels regulate intrinsic coronary vascular resistance in response to local metabolic changes. When myocardial oxygen demand is increased, endocardial vessels dilate in response to local metabolic factors, resulting in a regional increase in myocardial blood flow and, thereby, providing increased oxygen to these metabolically active tissues.

**Angina pectoris** (Fig. 23-5) is a clinical manifestation of chronic coronary artery disease. This symptom is characterized by precordial pressure-like discomfort resulting from **myocardial ischemia.** Most patients with chronic CAD demonstrate **stable angina,** a clinical syndrome in which ischemic chest pain occurs at characteristic workloads (e.g., walking on a treadmill). Pathologically, chronic coronary artery disease is associated with subintimal deposition of atheromas in the epicardial coronary arteries. In general, atherosclerotic plaques in patients with chronic stable angina are characterized by an overlying fibrous cap that is thick and resistant to disruption.

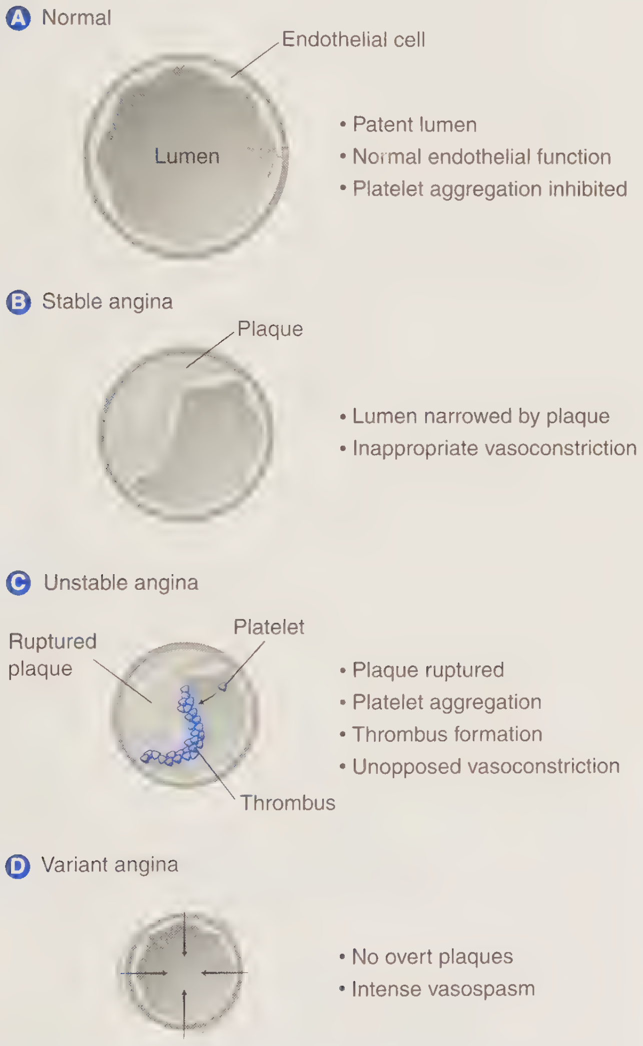


**Figure 23-5. Pathophysiology of Anginal Syndromes.** **A,** Normal coronary arteries are widely patent, the endothelium functions normally, and platelet aggregation is inhibited. **B,** In stable angina, atherosclerotic plaque and inappropriate vasoconstriction (caused by endothelial damage) reduce the vessel lumen diameter and, hence, coronary blood flow. **C,** In unstable angina, rupture of the plaque triggers platelet aggregation, thrombus formation, and vasoconstriction. Depending on the anatomic site of plaque rupture, this process can progress to non-Q wave (non-ST elevation) or Q wave (ST elevation) myocardial infarction. **D,** In variant angina, atherosclerotic plaques are absent, and ischemia is caused by intense vasospasm.

The immediate cause of angina pectoris is *an imbalance between myocardial oxygen supply and demand.* Under normal physiologic conditions, the diameter of the coronary lumen is modulated carefully to ensure adequate tissue perfusion in response to varying levels of myocardial oxygen demand. This ability to modulate blood flow is referred to as the **coronary flow reserve:**

$$\textbf{CFR = maximal CBF/resting CBF}$$

where CFR is coronary flow reserve, and CBF is coronary blood flow. In healthy individuals, the maximal CBF is approximately five-fold greater than the resting CBF. Because of this wide safety margin, the resting CBF does not decrease until an epicardial stenosis exceeds 80% of the original arterial diameter. Changes in maximal CBF can be observed more easily with exercise, because maximal CBF begins to decrease when an epicardial stenosis exceeds 50% of the original arterial diameter. Furthermore, in patients with chronic epicardial CAD, the coronary vasodilator capacity may be impaired becauses of endothelial dysfunction (discussed below), resulting in a further reduction in CFR. The overall decrease in CFR is directly related to the severity of epicardial stenosis. During periods in which myocardial oxygen demand exceeds CFR, demand-related ischemia occurs, and the patient experiences angina pectoris.

The degree of epicardial vessel stenosis and the degree of compensatory endocardial vessel dilation determine the hemodynamic consequence of an atherosclerotic plaque (Fig. 23-6). If the endocardial vessels are normal, then a stenosis that narrows the diameter of the vessel lumen by less than 50% does not significantly affect maximal coronary blood flow. However, if the stenosis narrows the lumen diameter by more than 80%, then the endocardial vessels must dilate to provide adequate perfusion to the myocardium at rest. The resting increase in endocardial vessel dilation occurs at the expense of coronary flow reserve, and the reduction in coronary flow reserve leads to inadequate myocardial blood flow at times of hyperemic stress. Myocardial ischemia can occur at rest when the stenosis exceeds 90% of the lumen diameter, because the endocardial vessels cannot maintain adequate myocardial perfusion even at maximal dilation.

## Endothelial Cell Dysfunction

**Endothelial dysfunction** is a general term for pathologic endothelial cell regulation. Clinically, endothelial dysfunction is manifested by abnormal vascular tone and prothrombotic properties.

Abnormal vascular tone is a result of dysregulated endothelial control of smooth muscle contraction: Arterial beds with endothelial dysfunction cannot relax in response to vasodilatory stimuli. For example, when mental stress or physical exertion trigger activation of the sympathetic nervous system (SNS), two opposing forces act on the coronary vascular endothelium: catecholamine-mediated vasoconstriction and nitric oxide (NO)-mediated vasodilation. Normally, release of

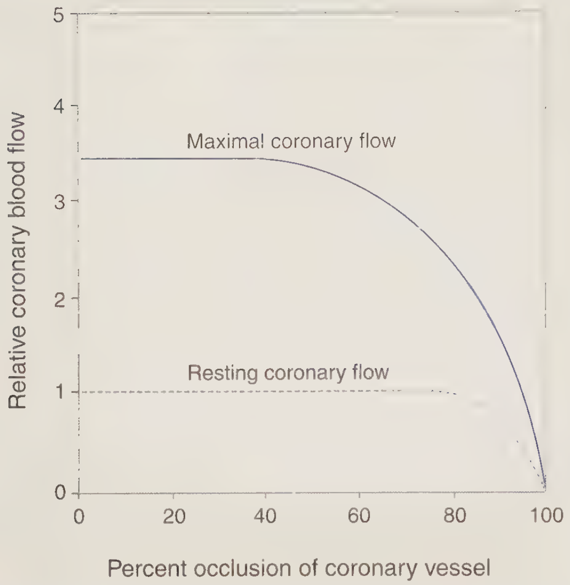


**Figure 23-6. Effect of Coronary Artery Occlusion on Resting and Maximal Coronary Blood Flow.** The dotted line depicts resting coronary blood flow, and the solid line represents maximal blood flow when there is full dilation of distal coronary vessels. Comparison of these two lines shows that maximal coronary blood flow is compromised when the lesion occludes more than about 50% of the vessel lumen, whereas, resting coronary blood flow is relatively unaffected until the lesion exceeds about 80% of the arterial diameter. The y-axis represents coronary blood flow relative to the flow in a resting coronary vessel with 0% occlusion.

NO by the coronary vascular endothelium is stimulated by the shear stress on the endothelium that results from increased blood flow. Eventually, the vasodilator effects of NO predominate over the vasoconstrictor effects of SNS activation, and the overall effect is coronary vasodilation. However, when the vascular endothelium is damaged, decreased production of endothelial vasodilators results in overwhelming catecholamine-mediated vasoconstriction.

Because the endothelium also plays crucial roles in regulating platelet activation and the coagulation cascade, endothelial cell dysfunction can promote blood coagulation (thrombosis) at the site of endothelial injury. Endothelial-derived NO and prostacyclin exert significant antiplatelet effects, and molecules on the surface of healthy endothelium have significant anticoagulant properties (see Chapter 21). Endothelial damage results in a decrease in these antiplatelet and anticoagulant mechanisms, leading to a local predominance of procoagulant factors and increasing the likelihood of platelet activation and initiation of the coagulation cascade.

## ACUTE CORONARY SYNDROMES

Acute coronary syndromes are most often caused by the fissuring or rupture of atherosclerotic plaques at sites of endothelial injury. Clinically, rupture of an atherosclerotic plaque is often characterized by **unstable angina,** which is defined as ischemic chest pain at rest, new onset anginal pain, or acceleration of the frequency or severity of anginal chest pain.

Plaque rupture leads to platelet activation and to activation of the coagulation cascade. Under physiologic circumstances, hemostasis at a site of vascular injury is self-limited by endogenous anticoagulant mechanisms (see Chapter 21). However, the dysfunctional endothelium at an atherosclerotic plaque site cannot elaborate sufficient anticoagulant factors to control the extent of clot formation. Unregulated coagulation can result in intraluminal thrombus formation. If the intraluminal thrombus occludes the epicardial coronary vessel at the site of plaque rupture, then blood flow beyond the point of obstruction ceases. Persistent, total epicardial vessel occlusion for more than several hours produces an acute myocardial injury that progresses inexorably to **transmural myocardial infarction.** The clinical presentation of this syndrome is variable, but out-of-hospital sudden cardiac death is a frequent occurrence (~30% of patients). In most cases, sudden cardiac death is caused by ischemia-induced electrical instability of the myocardium. In the absence of fatal electrical instability, transmural myocardial infarction typically presents with unremitting chest discomfort and ST segment elevation on the 12-lead ECG.

Plaque rupture leading to complete occlusion of a coronary artery often results in myocardial infarction and cardiovascular death. However, the thrombus is not always completely occlusive. When subtotal occlusion of a coronary artery occurs, the extent of ischemic necrosis is decreased, and the clinical syndromes of non-ST elevation (non-Q wave) myocardial infarction or unstable angina can result.

**Subendocardial** or **nontransmural myocardial infarction** (also called non-ST elevation or non-Q wave myocardial infarction) involves only the innermost layers of the myocardium. These myocardial regions are subjected to elevated intraventricular pressures as well as the biomechanical stresses that occur during cardiac contraction. As a result, the requirement for oxygen and nutrients is substantial in these regions, and the cardiomyocytes in these regions are particularly susceptible to ischemic injury. Patients with non-Q wave MI remain at risk for recurrence of ischemia, due to the presence of a persistent prothrombotic surface at the site of plaque rupture.

In contrast to non-Q wave MI, **unstable angina** refers to clinical presentations characterized by ischemic chest pain at rest, new-onset anginal pain, or the abrupt onset of a rapidly progressive anginal syndrome (Fig. 23-5). Enzymatic evidence of tissue infarction is absent, but patients with unstable angina are at high risk for myocardial infarction because of the presence of an active prothrombotic surface at the site of plaque rupture.

The extent of myocardial necrosis following ischemic injury depends on the mass of myocardium supplied by the occluded vessel, the duration of total occlusion of the vessel, and the degree of collateral circulation that is available to the border segments of the infarct territory. Regions of the myocardium supplied directly and exclusively by the occluded artery sustain extensive ischemic injury. Cell death occurs in a "wavefront," which progresses both spatially and temporally from the subendocardial to the epicardial surface of the myocardium. As a result, the extent of "transmurality" of a myocardial infarction bears a direct relationship to the duration of vessel occlusion. Adjacent to the region of transmural necrosis, a border zone of myocardium receives nutrients and oxygen from collateral vessels; this collateral perfusion alters the temporal progression of tissue infarction and can maintain the viability of border-zone cells for some period of time. However, in the absence of reperfusion of the occluded (infarct-causing) artery, lethal cardiomyocyte injury eventually occurs in these border-zone regions as well.

## CLINICAL MANAGEMENT OF ISCHEMIC HEART DISEASE

As noted above, the clinical approach to ischemic heart disease depends on whether the patient has chronic CAD or an acute coronary syndrome. Because chronic CAD results from an imbalance between myocardial oxygen supply and demand, treatment of chronic CAD focuses on re-establishing this balance, usually by reduction of oxygen demand. In comparison, treatment of acute coronary syndromes relies on re-establishing and maintaining the patency of the epicardial coronary artery. Both clinical presentations also require modification of underlying risk factors, including aggressive lipid-lowering therapy.

## CHRONIC CORONARY ARTERY DISEASE

Although the treatment goal in chronic stable angina is to "restore" the balance between myocardial oxygen supply (coronary blood flow) and myocardial oxygen demand (myocardial oxygen consumption), pharmacologic modulation of this dysequilibrium is *most often achieved via reduction of myocardial oxygen demand.* Because heart rate, contractility, and ventricular wall stress are the major determinants of myocardial oxygen demand, antianginal drugs can be categorized on the basis of their impact on these parameters.

### β-Adrenoceptor Antagonists

Activation of $\beta_1$-adrenergic receptors by the sympathoadrenal system leads to an increase in heart rate, contractility, and conduction velocity through the atrioventricular (AV) node. It, therefore, follows that antagonists acting at β-adrenergic receptors would decrease sinus rate, reduce inotropic state, and slow AV nodal conduction. *β-Blockers reduce myocardial oxygen demand by decreasing heart rate and contractility.* The drug-induced decrease in heart rate may also increase coronary blood flow via prolongation of the diastolic filling time.

β-Blockers (Table 23-3) are the cornerstone of medical treatment regimens in patients with chronic stable angina. When used in such patients, β-blockers decrease the peak heart rate achieved during exercise and delay the time to onset of angina. Dosing regimens for β-blockers are drug specific, reflecting the characteristic pharmacokinetics of each individual agent. As a general rule, the dose of drug is calibrated to maintain the resting heart rate at approximately 50 beats/minute and to maintain the peak heart rate during exertion at approximately 110–120 beats/minute.

β-Blockers are frequently coadministered with organic nitrates in patients with stable angina. This combination often is more effective than either agent used alone. β-Blockers are also frequently combined with calcium channel blockers (CCBs)—typically, with agents of the dihydropyridine class (see below). In early clinical trials, short-acting formulations of the dihydropyridine CCB nifedipine were associated with reflex tachycardia when administered as monotherapy; this tachycardia was attenuated when nifedipine was coadministered with a β-blocker. In current practice, the availability of long-acting dihydropyridine agents has effectively eliminated this side effect.

Extreme caution should be exercised when combining β-blockers with CCBs of the nondihydropyridine classes (e.g., diltiazem or verapamil). Coadministration of these drugs with β-blockers can result in synergistic suppression of SA nodal automaticity (leading to extreme sinus bradycardia) and/or AV nodal conduction (leading to high-grade AV conduction block). When used in the treatment of the rare patient with pure vasospastic or **variant angina** (i.e., no evidence of epicardial vessel obstruction, see Fig. 23-5), β-blockers can *induce* coronary vasospasm as a consequence of unopposed α-receptor–mediated vasoconstriction.

Because of their depressant effects on nodal tissues, β-blockers may be contraindicated in patients with symptomatic bradycardia and/or high-grade AV block. However, given the clear and consistent mortality benefit associated

**TABLE 23-3** Pharmacodynamic Properties of β-Blockers in Clinical Use

| DRUG | β-ADRENOCEPTOR SELECTIVITY | PARTIAL AGONIST ACTIVITY AT β-ADRENOCEPTORS |
|---|---|---|
| Propranolol<br>Nadolol<br>Timolol | Nonselective | No |
| Metoprolol<br>Atenolol<br>Betaxolol<br>Bisoprolol<br>Esmolol | $\beta_1 > \beta_2$ | No |
| Labetalol<br>Pindolol | Nonselective | Yes |
| Acebutolol | $\beta_1 > \beta_2$ | Yes |

with β-blockers in secondary prevention trials, it is currently standard clinical practice to implant a permanent transvenous pacing device if these rhythm abnormalities are the major contraindication to the use of β-blockers. (Secondary prevention trials test the efficacy of interventions aimed at preventing further cardiovascular disease in patients with known CAD.)

Although β-blockers are now being used in patients with clinically stable congestive heart failure (see below), it must be emphasized that the survival benefit demonstrated in CHF treatment trials occurred when these agents were initiated during periods of clinical stability. *β-Blockers must not be administered to patients who present with decompensated CHF.*

Asthma and chronic airway obstruction are common noncardiac disease states in which β-blockers are contraindicated. In patients with chronic airway obstruction, the decision to exclude β-blockers should be based on objective documentation of reversible airflow obstruction. Peripheral vascular disease is a relative contraindication to β-blocker therapy; the concern in this circumstance is the potential for antagonism of the $\beta_2$-adrenergic receptors that mediate dilation of peripheral vessels. In clinical practice, however, this concern seems to be primarily theoretical.

Commonly experienced subjective side effects of β-blockers include fatigue, lethargy, insomnia, and impotence. Although the mechanism of fatigue is unclear, the inability to perform exercise is directly related to drug-induced blunting of the physiologic tachycardia of exercise. Inhibition of $\beta_2$-adrenoceptor–mediated peripheral vasodilation is responsible for the impotence reported by 1% of patients treated with β-blockers.

## $Ca^{2+}$ Channel Blockers

Calcium channel blockers decrease the influx of calcium through voltage-gated L-type calcium channels in the plasma membrane. The resulting decrease in intracellular calcium concentration leads to reduced contraction of both cardiac myocytes and vascular smooth muscle cells (see Chapter 20).

Calcium channel blockers (CCBs) decrease myocardial oxygen demand, and may also increase myocardial oxygen supply. *CCBs decrease myocardial oxygen demand by decreasing systemic vascular resistance and decreasing cardiac contractility.* In the periphery, calcium entry into vascular smooth muscle cells is required for contraction of the cells and for the resulting increase in vasomotor tone. Calcium channel blockers prevent calcium entry, thereby causing relaxation of vascular smooth muscle and decreasing systemic vascular resistance. Calcium channel blockers can theoretically increase myocardial oxygen supply by blocking calcium-mediated increases in coronary vasomotor tone; the resulting dilation of epicardial vessels and arteriolar resistance vessels can theoretically increase coronary blood flow. However, the contribution of this coronary vasodilator mechanism to the clinical effects of the CCBs is controversial because the regional metabolic abnormalities that result from myocardial ischemia should effect a maximal vasodilator response, even in the absence of pharmacologic modulation.

The three classes of calcium channel blockers have differential negative inotropic effects on cardiac myocytes. Compared to verapamil and diltiazem, dihydropyridines (such as nifedipine) are more selective for calcium channels in the peripheral vasculature. All CCBs, however, do have the potential to impair contractile function by reducing intracellular calcium levels in cardiac myocytes.

Calcium channel blockers have been reported to be as effective as β-blockers in the treatment of chronic stable angina. If the initial treatment of angina with β-blockers alone is not successful, the calcium channel blockers can be used either in combination with β-blockers or as monotherapy. Calcium channel blockers seem to produce a greater antianginal effect when coadministered with β-blockers than when administered alone, although combination therapy can induce arrhythmias (see above). Despite this proven efficacy in reducing symptoms, no data yet support a mortality benefit associated with CCB therapy in patients with CAD, either as primary or secondary prevention.

Unlike the β-blockers, *calcium channel blockers are effective in the treatment of vasospastic angina.* Calcium channel blockers relieve the vasospasm of coronary vessels by dilating both epicardial coronary arteries and arteriolar resistance vessels.

Decompensated heart failure is a contraindication to the use of CCBs because of the negative inotropic effects of these drugs. However, newer-generation vasoselective dihydropyridines, such as amlodipine and felodipine, are well tolerated by patients with reduced left ventricular (LV) ejection fractions and can, therefore, be administered to patients with LV dysfunction and refractory angina. Even so, long-term treatment with CCBs in patients with congestive heart failure has failed to demonstrate a survival benefit. Because CCBs cause peripheral vasodilation, hypotension and peripheral edema can occur. Nonspecific actions of CCBs in the central nervous system and skin can lead to side effects of headache, dizziness, and flushing.

## Nitrates

Organic nitrates exert their principal therapeutic effect by reducing myocardial oxygen demand. Nitrate-induced dilation of the peripheral capacitance veins decreases preload and, thereby, reduces myocardial oxygen demand (see Chapter 20). Some investigators argue that nitrates also increase myocardial blood flow by reducing coronary vasomotor tone, although the magnitude of the incremental vasodilator effect in patients with regional myocardial ischemia is controversial. Nitrates may have a coronary vasodilator effect in patients with vasospastic angina. These agents also have antiaggregatory effects on platelets.

Nitrates improve exercise tolerance when used as monotherapy in patients with *stable exertional angina.* In patients with stable angina, nitrates have an additive antianginal effect in combination with β-blockers or CCBs. Sublingual nitroglycerin tablets or nitroglycerin sprays are effective for immediate relief of exertional angina. Provided that sufficient nitrate-free intervals are allowed (to delay the development of tolerance), long-acting nitrates (e.g., isosorbide dinitrate and mononitrate) are effective for prophylaxis and treatment of exertional angina.

The development of tolerance is the major obstacle to long-term use of nitrates. Through uncertain mechanisms (see Chapter 20), tolerance develops to both vasodilator and antiplatelet effects of these drugs. Dosing regimens that are punctuated by sufficiently long nitrate-free intervals (8–12 hours) may prevent nitrate tolerance. Headache, the most common side effect of nitrate therapy, can develop as a result of cerebral vessel dilation.

Nitrates are also effective in the treatment of both acute and chronic left ventricular failure. This treatment effect is related to the powerful venodilator action of the nitrates, an effect that results in peripheral redistribution of intravascular volume and marked reduction of preload. The anti-ischemic effect of the nitrates may be of particular value in patients with ischemia-related diastolic dysfunction. In this clinical setting, nitrates may effect both preload reduction and restoration of normal diastolic chamber compliance and filling.

### Aspirin

Because platelet activation is critically important in the initiation of thrombus formation (see Chapter 21), antiplatelet agents are useful prophylactic therapies for patients with CAD. Aspirin irreversibly inhibits platelet cyclooxygenase, an enzyme required for generation of the pro-aggregatory compound thromboxane $A_2$ ($TxA_2$). Platelets cannot regenerate cyclooxygenase, and the platelet inhibition that follows aspirin administration lasts for the lifespan of the platelet (approximately 10 days).

Unless specific contraindications are present, aspirin is a standard therapy for patients with chronic CAD. Aspirin is used to prevent arterial thrombosis leading to stroke and transient ischemic attack as well as myocardial infarction. It is most effective as a selective antiplatelet agent when taken at *low doses and/or infrequent intervals* (e.g., 81 mg once daily; see Chapter 21). Clinical data have demonstrated a significant treatment benefit for aspirin in patients with unstable angina (~50% reduction of death and nonfatal myocardial infarction). Aspirin is contraindicated in patients with a known allergy to the agent and should be used with caution in patients with synthetic dysfunction of the liver (bleeding diathesis). For patients with a history of peptic ulcer disease, aspirin suppositories can be used. The gastrointestinal side effects of aspirin can also be alleviated by coadministration of agents that decrease gastric acid production.

### Lipid-Lowering Agents

Clinical studies indicate that, in patients with known CAD, the administration of drugs that lower serum LDL-cholesterol decreases the risk of ischemic cardiovascular events. Refer to Chapter 22, Pharmacology of Cholesterol and Lipoprotein Metabolism, for a detailed discussion of lipid-lowering agents. The selection of a specific lipid-lowering agent is based on both clinical trial data and the patient's lipid phenotype.

HMG CoA reductase inhibitors are the most frequently used lipid-lowering agents. Because HMG CoA reductase mediates the first committed step in sterol biosynthesis, inhibitors of HMG CoA reductase dramatically reduce the extent of hepatic cholesterol synthesis. This reduction in cholesterol synthesis results in increased hepatic LDL receptor expression and, thereby, in increased clearance of cholesterol-containing lipoprotein particles from the bloodstream. Clinical trials (e.g., the Scandinavian Simvastatin Survival Study and the Cholesterol and Recurrent Events Study) indicate that lipid-lowering therapy reduces cardiovascular event rates in CAD patients who have mild-to-moderate elevations of LDL cholesterol. Dietary and other lifestyle modifications should also be included as part of a comprehensive approach to primary and secondary prevention. HMG CoA reductase inhibitors are contraindicated in women who are or may become pregnant or who are nursing.

## UNSTABLE ANGINA

Unstable angina may occur as the first presentation of coronary artery disease, in the early convalescent phase following myocardial infarction, or, in some cases, in patients with a history of stable CAD. (In the latter case, management strategies appropriate for unstable angina take precedence over those for stable CAD.) It has been estimated that, in patients with untreated unstable angina, there is a 15%–20% risk of progression to acute myocardial infarction (MI) over a period of 4–6 weeks. Aggressive treatment can reduce the risk of progression to acute MI by more than 50%.

The treatment goals in unstable angina are to relieve ischemic symptoms and to prevent additional thrombus formation at the site of plaque rupture. Unstable angina is typically treated with aspirin, heparin, and other antithrombotic drugs (e.g., platelet GPIIb–IIIa receptor antagonists) to prevent additional thrombus formation. Although conventional antianginal drugs have no demonstrable impact on mortality in unstable angina, these "demand-based" agents are also used empirically. *Thrombolytic agents are contraindicated in patients with unstable angina:* Use of these agents in unstable angina has been associated with a significant increase in morbidity and a trend toward increased mortality. If ischemic chest discomfort recurs after initiation of treatment for unstable angina, urgent coronary angiography is warranted (with revascularization guided by the angiographic data).

### Antianginal Drugs

Intravenous nitroglycerin is administered for the first 24 hours after the onset of unstable angina. The intravenous formulation is used to achieve and maintain predictable blood levels of the drug. After 24 hours, the asymptomatic patient can be switched to a long-acting oral nitrate preparation. To reduce the risk of subsequent MI, myocardial oxygen demand should also be reduced by coadministration of a β-adrenergic antagonist. Although $Ca^{2+}$ channel blockers such as verapamil and diltiazem also reduce myocardial oxygen demand, their use is purely palliative; unlike β-blockers, these agents have not been shown to reduce the risk of MI or cardiac death in patients with unstable angina.

### Heparin and Aspirin

In patients with unstable angina, heparin and aspirin reduce the risk of progression to myocardial infarction or cardiovascular death by ~50%. Although these agents do increase the risk of bleeding, the clinical benefits outweigh the potential side effects. Heparin and aspirin, when used in combination, seem to be more effective than either agent used alone in reducing cardiac mortality and recurrent ischemia.

## Glycoprotein IIb–IIIa Antagonists

Glycoprotein IIb–IIIa (GPIIb–IIIa) antagonists are highly efficacious antiplatelet agents. During platelet aggregation, GPIIb–IIIa receptors on activated platelets bind the bridging molecule fibrinogen. GPIIb–IIIa antagonists interfere with this critical step of platelet aggregation and, thereby, limit the size of the platelet plug. The use of GPIIb–IIIa antagonists has increased dramatically in recent years, both in the cardiac catheterization laboratory (during percutaneous revascularization procedures) and in the pharmacologic treatment of unstable angina. GPIIb–IIIa antagonists are effective in reducing the risk of fatal and nonfatal MI in patients with unstable angina. In addition to aspirin and heparin, a GPIIb–IIIa antagonist should be administered to patients with ongoing ischemia; eptifibatide and tirofiban have been approved for this use. The use of abciximab has been largely restricted to the periprocedure setting (i.e., in preparation for and immediately following percutaneous coronary intervention).

# ACUTE MYOCARDIAL INFARCTION

The three thrombolytic agents currently used in the pharmacologic management of acute myocardial infarction are streptokinase, APSAC (anisoylated plasminogen streptokinase activator complex), and recombinant tissue-type plasminogen activator (t-PA). (All are discussed in greater detail in Chapter 21.) One crucial factor that determines the success of thrombolytic therapy in acute MI is the *timeliness of administration.* It has been observed that patients who receive thrombolytic therapy within 2 hours of the onset of symptoms have a *2-fold improvement in survival rate* as compared to patients who receive thrombolytic therapy 6 hours after the onset of symptoms. This observation is consistent with the known relationship between the duration of vessel occlusion and the extent of transmural infarction.

## Streptokinase

The pharmacologic action of streptokinase involves two steps—complexation and cleavage. In the complexation reaction, streptokinase forms a stable, noncovalent 1:1 complex with plasminogen (either free plasminogen or fibrin-bound plasminogen). The complexation reaction produces a conformational change that exposes the active site on plasminogen. Plasminogen, now with its active site exposed, can effect proteolytic cleavage of *other* plasminogen molecules (again, either free plasminogen or fibrin-bound plasminogen) to plasmin and, thereby, initiate thrombolysis.

In the treatment of acute MI, streptokinase is administered as an intravenous loading dose followed by a continuous intravenous infusion. After 90 minutes of infusion, streptokinase produces, on average, 60% lumen patency in the previously occluded coronary vessel. However, the usefulness of streptokinase is limited by two factors. First, streptokinase is a foreign protein that is capable of eliciting antigenic reactions upon repeated administration. Patients with antibodies against streptococcus (from either a previous streptococcal infection or previous treatment with streptokinase) can develop an allergic reaction and fever. Second, because the streptokinase:plasminogen complex activates both fibrin-bound and free plasminogen molecules, its relatively nonspecific antithrombotic activity can result in systemic fibrinolysis.

## Anisoylated Plasminogen Streptokinase Activator Complex (APSAC)

APSAC is a preformed complex of streptokinase and plasminogen that is acetylated at the active site of the plasminogen. This acetylation blocks the enzymatic activity of plasminogen. APSAC is, therefore, administered as an inactive complex. The circulating complex binds to fibrin with high affinity and specificity, via the fibrin-binding site on the plasminogen component of the complex. The complex then undergoes spontaneous hydrolysis, releasing the acyl group from the plasminogen active site and resulting in activation of APSAC *at the site of the fibrin clot.* APSAC is administered as a single intravenous injection over a 5-minute period. Compared to streptokinase, APSAC exhibits greater selectivity for fibrin clots and more thrombolytic activity. However, the selectivity of APSAC for fibrin clots is not complete, and a systemic lytic state may occur in some patients.

## Recombinant Tissue-Type Plasminogen Activator (t-PA)

Recombinant tissue-type plasminogen activator (t-PA) is effective in restoring the patency of occluded coronary arteries, limiting cardiac dysfunction, and reducing mortality following acute myocardial infarction. As with endogenously produced t-PA, recombinant t-PA binds to newly formed thrombi with high affinity, causing fibrinolysis at the site of a thrombus. Once bound to the nascent thrombus, t-PA undergoes a conformational change that renders it a potent activator of plasminogen. t-PA is a poor activator of plasminogen in the absence of fibrin binding.

Recombinant t-PA is typically administered intravenously at a high dose rate for 1 hour and then at a lower dose rate for the next 2 hours. Despite its high affinity for fibrin-bound plasminogen, recombinant t-PA at pharmacologic doses can (as with other thrombolytic agents) generate a systemic lytic state and cause undesirable bleeding, including cerebral hemorrhage. Thus, this agent is contraindicated in patients who have had a recent stroke.

## Postmyocardial Infarction Management

After a myocardial infarction, a patient must be carefully managed to prevent re-infarction. The goals of any post-MI medical regimen are 2-fold: (1) preventing and treating residual ischemia; and (2) identifying and treating such major risk factors as hypertension, smoking, hyperlipidemia, and diabetes mellitus. Because the extent of myocardial infarction and its functional consequences vary greatly among patients, the medical regimen must be individualized. However, the American College of Cardiology and the American Heart Association have made the following general recommendations for the management of post-MI patients:

1) Aspirin (75–325 mg/d), in the absence of contraindications (clopidogrel for patients with a contraindication to aspirin)
2) β-Blockers, in the absence of contraindications

3) Lipid-lowering agents (target LDL cholesterol, <100 mg/dL)
4) ACE inhibitors for patients with congestive heart failure, left ventricular dysfunction (ejection fraction, <40%), hypertension, or diabetes.

In addition to designing an individualized drug regimen, the physician must also educate the patient about risk factors for the recurrence of MI. A useful mnemonic for guiding overall treatment in post-MI patients is **ABCDE** (**A**spirin, ACE inhibitors, and antianginals; **B**eta-blockers and blood pressure; **C**holesterol and cigarettes; **D**iet and diabetes; **E**ducation and exercise).

### ■ Case, Part III: Congestive Heart Failure

Mr. Nandrunson is discharged from the hospital on a multidrug regimen that includes aspirin, clopidogrel, metoprolol, atorvastatin, and captopril. He does well as he increases his activity level during the first 4–6 weeks after the infarction. At that point, however, he once again experiences breathlessness at moderate levels of exertion. He initially attributes this to deconditioning, but becomes concerned when he wakes from sleep with severe breathlessness in the early morning hours. He schedules an appointment with his physician for later that day.

On exam in the physician's office, Mr. Nandrunson appears comfortable seated in the upright position. His heart rate is 64 beats/min, and his blood pressure is 168/100 mm Hg. The pulmonic component of S2 is prominent (representing a change from his previous exams) and the apical S4 is again noted; there is a grade III/VI apical holosystolic murmur with radiation to the left axilla. An echocardiogram reveals akinesis of the basal segment of the inferior wall of the left ventricle, with more prominent thinning and aneurysmal remodeling of the segment. The left ventricular ejection fraction is 40%. Although the mitral valve leaflets and the supporting structures of the valve appear structurally normal, there is a degree of posterior leaflet prolapse (LV → LA) during ventricular systole. A Doppler study confirms the presence of mitral regurgitation that is at least moderate in severity. The right ventricle is dilated and hypertrophic, with relative preservation of systolic function. Repeat catheterization is performed to assess the etiology of the patient's new biventricular heart failure. Angiography shows wide patency of the right coronary artery at the site of the prior PTCA/stent intervention, and the left coronary artery is free of obstruction. Hemodynamic catheterization data demonstrate increased pulmonary artery and right ventricular pressures.

## QUESTIONS

■ **1.** What pharmacologic strategies are available to treat right heart failure?

■ **2.** What modifications should be made in Mr. Nandrunson's medication regimen to optimize treatment of his left ventricular failure?

■ **3.** What parenteral inotropic agents are available if his symptoms prove refractory to oral inotropes?

## PATHOPHYSIOLOGY OF CONGESTIVE HEART FAILURE

Heart failure is a common clinical problem. As many as 5 million patients in the United States carry this diagnosis, with approximately 500,000 new cases diagnosed each year. The syndrome of heart failure has a grave prognosis: the mortality rate at 5 years approximates 50%, and, in the subset of patients with the most severe clinical symptoms, annual mortality is as high as 30%–50%.

Because the impairment of contractile function that underlies this syndrome is often irreversible, congestive heart failure (CHF) is typically a chronic illness punctuated by episodes of acute decompensation. Management of CHF often requires the clinician to construct, evaluate, and modify a treatment regimen that includes multiple drugs, some of which may carry significant risk for adverse interactions. In addition, acute exacerbations are often multifactorial in etiology, with contributions from dietary indiscretion (excess sodium or fluid intake), nonadherence to prescribed medications, concomitant noncardiac illness, and myocardial ischemia and/or progression of the proximate cardiac disease. It is also important to consider the possibility that activation of neurohumoral regulatory systems are driving the clinical decompensation.

Although the discussion that follows emphasizes cardiogenic circulatory failure, it is important to recognize that circulatory failure can occur in the absence of cardiac pump dysfunction (Table 23-4). Common examples include abnormalities of cardiac filling (e.g., hypovolemia), cardiac rhythm (e.g., bradycardia or tachycardia), or the peripheral circulation (e.g., distributive shock related to sepsis).

## ETIOLOGIES OF CONTRACTILE DYSFUNCTION

Left ventricular contractile dysfunction **(systolic heart failure)** is the primary cause of congestive heart failure. Although there are multiple disease states that result in contractile dysfunction, the majority of cases of left heart failure (~70%) can be attributed to **coronary artery disease.** Additional causes of systolic heart failure include chronic abnormalities of the loading conditions imposed upon the heart, such as **systemic arterial hypertension** (pressure loading) and **valvular heart disease** (volume loading from mitral regurgitation or aortic insufficiency; pressure loading from aortic stenosis). In disease states associated with abnormal loading conditions, the contractile performance of the myocardium is initially preserved, but cardiomyocyte injury and whole-organ contractile dysfunction supervene if the abnormal loading conditions are not corrected. The latter phase of cardiac pump dysfunction has been referred to as **cardiomyopathy of chronic overload.** Systolic dysfunction can also result from diverse conditions in which the proximate pathologic abnormality is cardiomyocyte injury or dysfunction. Because the characteristic structural remodeling of the heart in these states of primary myocyte dysfunction is LV chamber dilation with or without wall thinning, these conditions are referred to as **dilated cardiomyopathies.**

TABLE 23-4 Causes of Circulatory Failure in the Absence of Cardiac Pump Dysfunction

| CAUSE OF CIRCULATORY FAILURE | MECHANISM |
|---|---|
| Abnormal cardiac filling | Hypovolemia (e.g., hemorrhage)<br>Cardiac tamponade (compression by pericardial fluid prevents normal diastolic filling) |
| Abnormal cardiac rhythm | Bradycardia (↓ rate → ↓ forward output)<br>Tachycardia (↑ rate → ↓ duration of diastolic filling interval) |
| Abnormal peripheral circulation | Hypertensive crisis (↑ SVR → ↑impedance to LV ejection → ↓ stroke volume)<br>Distributive shock (↓ SVR → ↓ MAP → organ hypoperfusion) |

SVR, systemic vascular resistance. MAP, mean arterial pressure.

Symptomatic heart failure can also occur in patients with normal or near-normal LV systolic function (i.e., preserved LV ejection fraction). In such cases, the symptoms of left heart failure are caused by abnormalities of left ventricular relaxation and/or filling **(diastolic heart failure)**. Impaired relaxation results in an elevation of LV diastolic pressure at any given filling volume. This elevation of LV diastolic pressure causes elevation of left atrial and pulmonary capillary pressures which, in turn, leads to transudation of fluid into the pulmonary interstitium (as well as secondary, or passive, elevation of pulmonary artery and right heart pressures). The most common cause of isolated diastolic heart failure is **acute myocardial ischemia.** In the setting of acute reversible ischemia (i.e., ischemia not associated with myocardial infarction), left ventricular diastolic pressures increase as a consequence of incomplete left ventricular relaxation. (Recall from the discussion in Chapter 18 that cardiomyocyte relaxation as well as contraction depend on adequate levels of intracellular ATP.)

Both systolic and diastolic heart failure can be understood by considering the determinants of cardiac performance and the pathophysiologic conditions that affect these parameters. Each of the principal factors affecting stroke volume—preload, afterload, and contractility—can be described by their effect on cardiac function curves. Figure 23-7 illustrates a normal left ventricular (LV) pressure–volume loop. In the normal cycle, LV volume increases when the mitral valve opens during diastole. Isovolumetric contraction begins when LV pressure exceeds left atrial pressure and the mitral valve closes; during this segment of the cycle, intraventricular pressure increases while intracavitary volume remains constant. Ejection begins, and the LV stroke volume is propelled into the aorta, when the impedance to LV ejection is exceeded and the aortic valve opens; ejected blood is then transmitted to the peripheral circulation by the elastic properties of the aorta. The aortic valve closes when LV pressure falls below aortic pressure; at this point, intraventricular pressure decreases rapidly (isovolumetric relaxation), up to (and perhaps beyond) the point at which the mitral valve opens, and the cycle is repeated.

As illustrated in Figure 23-8A, the forward stroke volume ejected by the left ventricle depends on the degree of LV filling during diastole, or **preload.** This fundamental relationship between preload and stroke volume is the **Frank–Starling law;** it derives from the relationship between muscle length

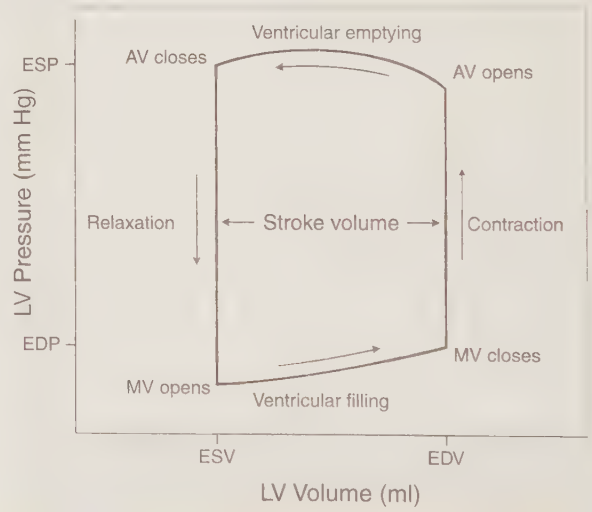


**Figure 23-7. Normal Left Ventricular Pressure–Volume Loop.** Mitral valve (MV) opening allows the left ventricular (LV) volume to increase as the chamber fills with blood during diastole. When ventricular pressure exceeds left atrial pressure, the mitral valve closes. During the isovolumetric phase of systolic contraction, the left ventricle generates a high pressure, which eventually forces open the aortic valve (AV). Ejection of the stroke volume ensues, and the aortic valve closes when aortic pressure exceeds LV pressure. Isovolumetric relaxation returns the ventricle to its lowest pressure state, and the cycle is repeated. Stroke volume (i.e., the volume of blood ejected with each contraction cycle) is the difference between end-diastolic volume (EDV) and end-systolic volume (ESV). EDP, end-diastolic pressure; ESP, end-systolic pressure.

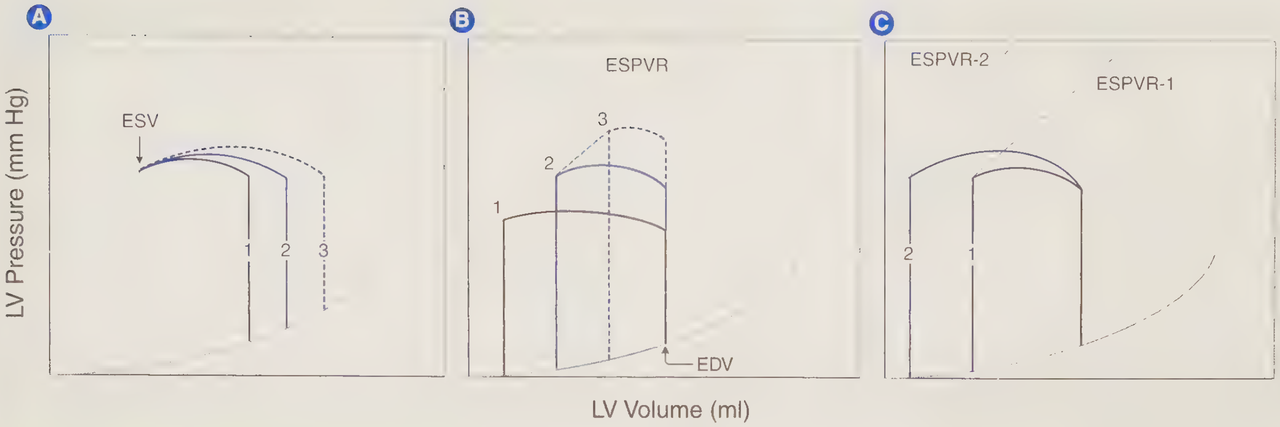


**Figure 23-8. Determinants of Cardiac Output.** Changes in preload, afterload, and myocardial contractility alter the pressure-volume relationship of the cardiac cycle. **A,** Increases in preload (lines 1, 2, 3) result in greater stretch of ventricular myocytes, development of greater ventricular pressure, and ejection of greater stroke volume (the Frank–Starling mechanism). Note that the end-systolic volume (ESV) is the same in each case, because the contractility of the heart has not changed. **B,** Increases in afterload (points 1, 2, 3) create greater impedance to left ventricular output and result in proportionately decreased stroke volume (the difference between end-diastolic volume (EDV) and ESV). The end-systolic pressure is linearly related to ESV; this linear relationship is called the end-systolic pressure-volume relationship (ESPVR). **C,** Increases in myocardial contractility (lines 1, 2), as occurs after administration of a positive inotrope, shift the ESPVR up and to the left, resulting in increased stroke volume.

and degree of muscle shortening, as described in Chapter 18. In brief, increased diastolic volume mechanically stretches the myocardium and, thus, stretches the myocytes. As a result, a higher fraction of the actin filament length is exposed in each sarcomere and is, thereby, available for myosin cross-bridge formation when the cardiomyocyte is depolarized.

Impedance to left ventricular ejection, or **afterload,** also determines the stroke volume (Fig. 23-8B). As impedance to ejection increases (increased afterload), the stroke output of the ventricle falls. This characteristic of the intact heart derives from the fact that increasing the resistance against which cardiac muscle must contract leads to a decrease in the extent of shortening (i.e., to reduced stroke volume). The sensitivity of stroke volume to outflow resistance is accentuated in the failing ventricle; this observation explains the ability of agents that decrease afterload to increase LV stroke volume (see below).

A third determinant of cardiac performance is **contractility,** also described in Chapter 18. The contractile state of the left ventricle is described by the end-systolic pressure-volume relationship (**ESPVR,** Fig. 23-8C). The ESPVR is, in effect, a variant of the Frank–Starling law. While the Frank–Starling law defines the relationship between LV diastolic volume (or preload) and LV stroke volume (or cardiac output), the ESPVR describes the relationship between diastolic filling volume and left ventricular tension development during isovolumetric contraction. As shown in Figure 23-8C, an increase in the contractile state of the left ventricle, reflected by an upward shift of the ESPVR, results in a greater degree of tension development for any given end-diastolic volume. In the presence of a fixed afterload, increased contractility results in a greater degree of muscle shortening and an increase in LV stroke volume.

A final determinant of cardiac pump performance is **heart rate.** However, if LV contractile performance is normal, then impairment of cardiac output occurs as a consequence of abnormal heart rate only at extreme rates outside the physiologic range.

## CARDIAC COMPENSATION

As the ability of the myocardium to maintain normal forward output fails, compensatory mechanisms are activated to preserve circulatory function. The Frank–Starling mechanism increases stroke volume in direct response to increased preload; this recruitment of preload reserve is the first response of the system to hemodynamic stress. Hemodynamic stress that cannot be fully compensated by the Frank–Starling mechanism stimulates signaling systems that initiate structural changes at the cellular level, a process referred to as **remodeling** of the myocardium. Although the underlying stimuli for remodeling remain an active area of investigation, it has been noted that the specific pattern of remodeling is determined by the nature of the applied stress. If the Frank–Starling mechanism and remodeling mechanisms are unable to re-establish adequate forward cardiac output, then neurohumoral systems are also activated. These systems modulate intravascular volume and vasomotor tone to maintain oxygen delivery to critical organs. Although each of these compensatory mechanisms does contribute to the maintenance of circulatory function, each may also contribute to the development and progression of pump dysfunction and circulatory failure, as described below.

### Frank–Starling Mechanism

As noted above, increased preload stimulates a direct increase in stroke volume in the intact heart via the Frank–Starling mechanism. Although this mechanism remains operative in the failing heart, *the relationship between end-diastolic volume and stroke volume is altered.* In patients with systolic dysfunction, the relationship between end-diastolic volume and stroke volume is characterized by a flatter plateau (Fig. 23-9). Although volume expansion can be a useful strategy for increasing stroke volume in patients operating on the ascending limb of the Starling curve, in fact, the majority of patients with heart failure operate with *elevated* intravascular volume that derives from the recruitment of preload reserve and from neurohormonal activation of the sympathoadrenal axis and the renin-angiotensin–aldosterone system (see below). Thus, the treatment of cardiogenic circulatory failure does not typically involve volume expansion.

## Cardiac Remodeling and Hypertrophy

In the setting of increased myocardial wall stress, ventricular systolic performance is maintained by the development of cardiac hypertrophy. Because left ventricular ejection fraction is inversely proportional to wall stress, adaptations that decrease wall stress increase LV ejection fraction. **Laplace's law** states that wall stress ($\sigma$) is directly proportional to the pressure ($P$) and radius ($R$) of a chamber, and inversely proportional to wall thickness ($h$):

$$\sigma = P \times R/2h \qquad \text{Equation 23-1}$$

In cases of chronic pressure overload, such as aortic stenosis or systemic hypertension, the left ventricle develops a **concentric** pattern of hypertrophy, as contractile proteins and new sarcomeres are added to cardiac myocytes in parallel to the existing myofilaments. This pattern simultaneously increases wall thickness ($h$) and decreases cavity size ($R$), resulting in a net reduction in systolic wall stress and, thereby, preserving systolic performance. The disadvantage of concentric remodeling derives from the *decrease in LV compliance* that occurs as a consequence of this pattern of hypertrophy. In a ventricle with reduced compliance, diastolic pressure in the hypertrophic chamber is increased at any given filling volume, predisposing to the development of congestive symptoms.

In conditions of chronic volume overload, such as mitral or aortic regurgitation, the left ventricle develops **eccentric pattern hypertrophy,** as contractile proteins and new sarcomeres are added in series to existing myofilaments. This pattern of hypertrophy helps to maintain cardiac performance via modulation of diastolic wall stress. In contrast to the situation that occurs after concentric remodeling, eccentric hypertrophy is associated with *increased LV compliance*. The increase in chamber compliance allows the LV diastolic volume to increase without a concomitant elevation of left ventricular and left atrial diastolic pressures. The ability of the left ventricle to continue preload recruitment without elevating intracardiac pressures allows the system to maintain forward cardiac output by a volume-driven increase in total stroke volume. During the compensated phase of eccentric pattern hypertrophy, LV wall thickness increases in approximate proportion to the increase in chamber radius.

## Neurohumoral Activation

Failure of the heart to provide adequate forward output recruits sympathetic nervous reflexes, with several deleterious consequences (Fig. 23-10). Decreased arterial pressure acti-

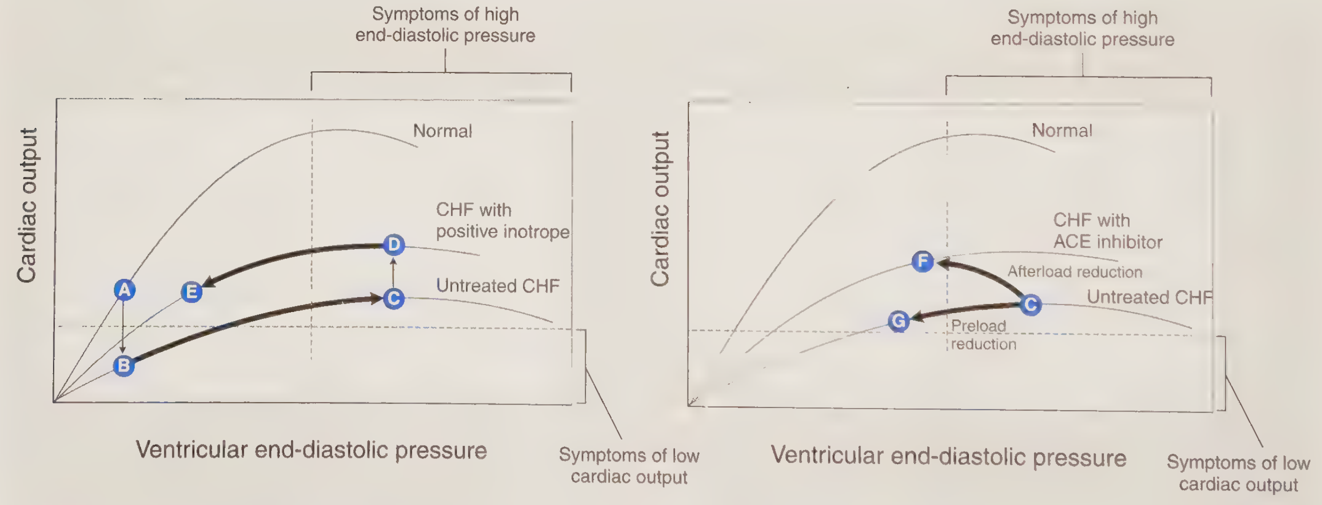


**Figure 23-9. The Frank–Starling Relationship in CHF. Left panel:** The normal Frank–Starling relationship shows a steep increase in cardiac output with increasing ventricular end-diastolic pressure (preload). Point A describes the end-diastolic pressure and cardiac output of a normal heart under resting conditions. With contractile dysfunction (untreated CHF), cardiac output falls (**B**) and the Frank–Starling curve flattens so that increasing preload translates to only a modest increase in cardiac output (**C**). This increase in cardiac output is accompanied by symptoms of high end-diastolic pressure, such as dyspnea. Treatment with a positive inotrope, such as digitalis, shifts the Frank–Starling curve upward, and cardiac output increases (**D**). The improvement in myocardial contractility supports a sufficient reduction in preload that the venous congestion is relieved (**E**). **Right panel:** Two of the principal pharmacologic treatments of CHF are afterload reduction (e.g., ACE inhibitors) and preload reduction (e.g., diuretics). Afterload reduction (**F**) increases cardiac output at any given preload, and, thus, elevates the Frank–Starling relationship. Preload reduction (**G**) decreases congestive symptoms by decreasing ventricular end-diastolic pressure along the same Frank–Starling curve.

vates the baroreflex to stimulate sympathetic release of catecholamines, producing tachycardia (via cardiac $\beta_1$ receptors) and vasoconstriction (via peripheral $\alpha_1$ receptors). Stimulation of $\beta_1$ receptors on renal juxtaglomerular (JG) cells promotes the release of renin. JG cells also release renin in response to the decreased renal perfusion that accompanies decreased cardiac output. Renin cleaves circulating angiotensinogen to angiotensin I, which is further converted by angiotensin converting enzyme (ACE) in the pulmonary circulation to angiotensin II (AT II). AT II acts through $AT_1$ receptors to increase arterial vasomotor tone. AT II also increases intravascular volume by activating several physiologic mechanisms, including the release of aldosterone from the adrenal glands (thus promoting salt and water retention), the release of vasopressin (ADH) from the posterior pituitary gland, and the activation of thirst centers in the hypothalamus. In addition, AT II seems to be an important mediator of vascular and myocardial hypertrophy.

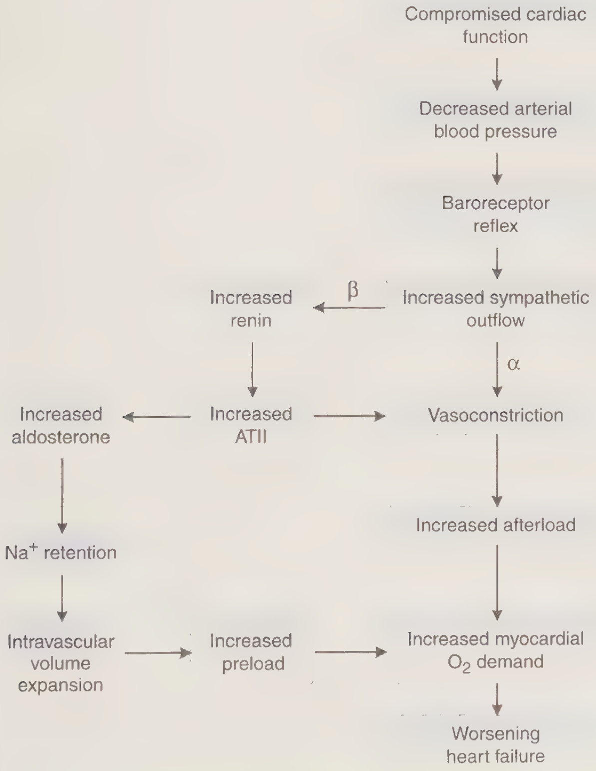


**Figure 23-10. Neurohumoral Effects of Congestive Heart Failure.** Compromised cardiac function leads to decreased arterial blood pressure, which activates baroreceptors that increase sympathetic outflow. α-Adrenergic sympathetic outflow causes vasoconstriction, an effect that increases afterload. The increased afterload creates a greater pressure against which the heart must contract, and, therefore, increases myocardial $O_2$ demand. β-Adrenergic sympathetic outflow increases juxtaglomerular cell release of renin. Renin cleaves angiotensinogen to angiotensin I, and angiotensin I is then converted to the active hormone angiotensin II (AT II). AT II has a direct vasoconstrictor action; it also increases aldosterone synthesis and secretion. Aldosterone increases collecting duct $Na^+$ reabsorption, leading to intravascular volume expansion and increased preload. Together, the increased afterload and preload increase myocardial $O_2$ demand. In the already compromised heart, these increased stresses can lead to worsening heart failure.

The tachycardia and increased intravascular volume that accompany activation of these neurohumoral mechanisms do help to maintain forward cardiac output, and the vasoconstriction that occurs allows the circulation to override local autoregulation of blood flow and, thereby, to maintain perfusion of critical organs. However, sympathetic stimulation of the heart also increases myocardial oxygen demand, as a result of the increased afterload (arteriolar constriction) and increased preload (retention of sodium and water) that accompany neurohumoral activation. Continued sympathetic stimulation eventually results in down-regulation of β-adrenergic receptors, further impairing the ability of the system to maintain forward output. The central aim of the current pharmacologic management of CHF is to modulate the action of these neurohumoral effectors (Fig. 23-11).

## CLINICAL MANAGEMENT OF CONGESTIVE HEART FAILURE

Pharmacologic treatment of CHF has improved dramatically over the past three decades. The ascent of "load-active" therapy has revolutionized the management of patients with CHF, and numerous large-scale clinical trials have demonstrated that these therapies are associated with statistically significant reductions in morbidity and mortality. In addition, improvements in the detection and treatment of hypertension and the management of complex multivessel CAD have dramatically altered the clinical course of patients with contractile dysfunction. It is helpful to organize the treatment strategies for contractile dysfunction in patients who either manifest, or are at risk for, symptomatic congestive heart failure according to the following pharmacologic targets: preload reduction, afterload reduction, and contractility enhancement. Table 23-5 provides a summary of the hemodynamic effects and mechanisms of action of the drug classes that are commonly used to treat congestive heart failure.

## PRELOAD REDUCTION

### Diuretics

Diuretics have long been a cornerstone of the pharmacologic management of patients with left heart failure and remain an integral component of the treatment of patients with congestive symptoms and/or intravascular volume overload. However, despite the efficacy of these agents in reducing congestive symptoms, there is no evidence of a mortality benefit from treatment with either loop diuretics or thiazide diuretics.

The natriuretic agents most commonly used in CHF are the loop diuretics, which include furosemide and bumetanide. These drugs inhibit the sodium/potassium/chloride cotransporter in the thick ascending limb of Henle, resulting in increased excretion of sodium, potassium, and water. Such thiazide diuretics as hydrochlorothiazide are also used to treat congestive symptoms, particularly in patients with hyper-

SCIENCES

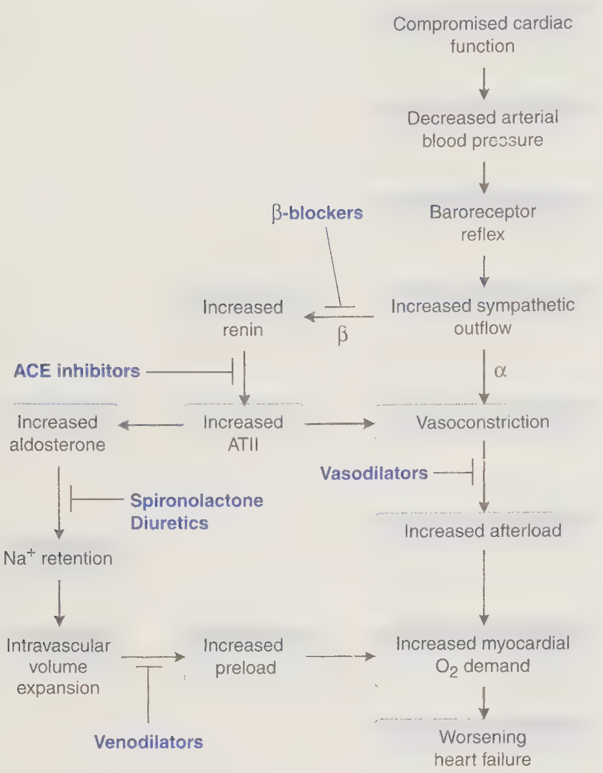


**Figure 23-11. Pharmacologic Modulation of the Neurohumoral Effects of CHF.** Many therapeutic agents used in the management of congestive heart failure modulate the neurohumoral systems that are activated by compromised cardiac function. The renin-angiotensin–aldosterone system can be inhibited by (1) β-adrenergic antagonists (β-blockers), which inhibit renin release by the juxtaglomerular cells of the kidney; (2) ACE inhibitors, which prevent the conversion of angiotensin I to the active hormone angiotensin II; and (3) spironolactone, which competitively inhibits aldosterone binding to the mineralocorticoid receptor. Diuretics promote $Na^+$ excretion and, thereby, counteract the $Na^+$ retention stimulated by activation of the renin-angiotensin–aldosterone system. Venodilators counteract the effect of intravascular volume expansion by increasing peripheral venous capacitance and, thereby, decreasing preload. Direct arterial vasodilators alleviate the α-adrenergic receptor-mediated and angiotensin II receptor-mediated vasoconstriction induced by increased sympathetic outflow. Cardiac glycosides, β-adrenergic receptor agonists, and cardiac phosphodiesterase inhibitors are also used in CHF to increase myocardial contractility (not shown).

tensive heart disease and LV systolic dysfunction. Thiazides inhibit sodium and chloride reabsorption in the distal convoluted tubule and are less efficacious natriuretic agents than the loop diuretics. Thiazides are often ineffective as monotherapy for congestive symptoms in patients with renal insufficiency. Thiazides are sometimes coadministered with loop diuretics in patients with reduced GFR and refractory volume overload, and in selected patients with CHF in whom treatment with loop diuretics alone does not achieve adequate diuresis. (Refer to Chapter 19 for a more extended discussion of diuretics.)

### Spironolactone

Spironolactone is a potassium-sparing diuretic that acts as a competitive antagonist at the aldosterone receptor to decrease sodium–potassium exchange in the distal tubule and collecting duct of the nephron. A recent clinical trial of this agent in CHF patients (the RALES study) has received much attention. In this study, patients with severe CHF were treated with low-dose spironolactone (25–50 mg daily); the CHF patients were free of significant renal impairment and were concomitantly receiving "standard" therapy for heart failure (ACE inhibitor, loop diuretic ± digoxin). In patients treated with spironolactone, all-cause mortality (including sudden cardiac death and death from progressive heart failure) was reduced by approximately 30%, as were hospital admissions for exacerbations of CHF. Because spironolactone is often administered in combination with an ACE inhibitor (see below), and both spironolactone and ACE inhibitors decrease $K^+$ excretion, it will be necessary to redefine safety criteria for plasma $K^+$ levels in patients with congestive heart failure who are treated with these agents.

### Venodilators

Venodilator agents are often coadministered with diuretics in patients with congestive symptoms. The prototypical venodilator is nitroglycerin (NTG); this drug increases venous capacitance and, thereby, decreases venous return to the heart. The decrease in venous return results in reduced LV chamber volume and reduced LV diastolic pressure. These effects of the nitrates decrease myocardial oxygen demand, which may be especially beneficial in patients with co-existing angina and left ventricular dysfunction.

Nitrates may also be particularly effective in cases where left heart failure results from acute myocardial ischemia. In this condition, left ventricular relaxation is impaired, LV compliance is decreased, and LV diastolic pressure is increased at any given chamber volume. By increasing venous capacitance, nitrates reduce venous return to the heart and decrease LV diastolic volume. In turn, the decrease in diastolic volume leads to a decrease in myocardial oxygen consumption, alleviation of the ischemia, and improved LV relaxation. Thus, the beneficial effects of nitrate administration in this setting include both preload reduction and improvement in LV compliance.

## AFTERLOAD REDUCTION

### ACE Inhibitors

By reversibly inhibiting angiotensin converting enzyme (ACE), ACE inhibitors reduce the production of angiotensin II (AT II) and, thereby, cause a number of clinically beneficial effects. AT II is an important component of the neurohumoral regulation of the failing circulation. In response to renal hypoperfusion, the kidney increases renin secretion, which results in increased production of AT II (see Chapter 19). In turn, AT II stimulates the adrenal gland to secrete aldesterone. Overall, activation of the renin-angiotensin–aldosterone system increases vasomotor tone as well as sodium and water retention. These hemodynamic alterations result in increased intravascular volume (leading, ultimately,

**TABLE 23-5** Pharmacologic Agents Used in the Treatment of Congestive Heart Failure

| DRUG OR DRUG CLASS | MECHANISM OF ACTION | HEMODYNAMIC EFFECT | CLINICAL NOTES |
|---|---|---|---|
| **Drugs with Proved Mortality Reduction** | | | |
| ACE inhibitors | Inhibit ATII generation $\rightarrow \downarrow$ $AT_1$ receptor activation | Decreased afterload<br>Decreased preload | May cause hyperkalemia |
| β-Blockers | Block β-adrenergic receptor activation $\rightarrow \downarrow$ renin release | Decreased afterload<br>Decreased preload | May be relatively contraindicated in severely decompensated heart failure |
| Spironolactone | Blocks aldosterone receptor activation | Decreased preload | Mortality benefit may be independent of hemodynamic effects; may cause hyperkalemia |
| **Drugs Used for Symptomatic Improvement** | | | |
| $Na^+/H_2O$ restriction | Decrease intravascular volume | Decreased preload | May help limit edema formation |
| Diuretics | Inhibit renal $Na^+$ reabsorption | Decreased preload | Furosemide most effective for treating congestive symptoms |
| Digoxin | Inhibit $Na^+/K^+$ ATPase $\rightarrow \uparrow$ intracellular $Ca^{2+}$ $\rightarrow \uparrow$ contractility | Increased contractility | Delays atrioventicular nodal conduction |
| Organic nitrates | Increase NO $\rightarrow$ venous smooth muscle relaxation $\rightarrow \uparrow$ venous capacitance | Decreased preload | Reduces myocardial $O_2$ demand |
| Dobutamine | Stimulates β-adrenergic receptors | Increased contractility ($\beta_1$ effect)<br>Decreased afterload ($\beta_2$ effect) | Used in the acute setting only |
| Amrinone, milrinone | Inhibit phosphodiesterase $\rightarrow \uparrow$ β-adrenergic effect | Increased contractility<br>Decreased afterload<br>Decreased preload | Used in the acute setting only |

to increased LV diastolic filling and increased LV stroke volume) and in peripheral redistribution of the cardiac output (mediated by the vasoconstrictor effects of AT II).

Administration of an ACE inhibitor reverses both the vasoconstriction and the volume retention that characterize renin-angiotensin–aldosterone system activation. The reduction in afterload decreases the impedance to LV ejection and increases LV stroke volume. The reversal of aldosterone-related volume retention decreases both intravascular volume and preload. These responses are synergistic in patients with CHF: As renal blood flow increases (because decreased systemic vascular resistance leads to increased stroke volume), GFR is also increased, resulting in increased delivery of sodium and water to the distal nephron, where (in the absence of a renin-stimulated elevation of aldosterone levels) natriuresis and diuresis occur. ACE inhibition can also increase venous capacitance (and, thereby, reduce preload) by decreasing the degradation of the endogenous vasodilator bradykinin. ACE inhibitors can provide further benefit in patients with concomitant CHF and CAD, by altering the remodeling of the myocardium that occurs after Q-wave infarction.

ACE inhibitors have been shown to have a statistically significant impact on survival in patients with heart failure. This mortality benefit was first demonstrated in patients with severe heart failure in the CONSENSUS trial: The mortality reduction approximated 40% at 6 months and 31% at 1 year. The mortality benefit of the ACE inhibitors was confirmed in a broader spectrum of patients in the SOLVD Treatment Trial (16% reduction in mortality) and the V-Heft II Trial (28% reduction in mortality), as well as in patients in the convalescent phase following myocardial infarction (SAVE Trial, 19% reduction in mortality).

Angiotensin receptor blockers ($AT_1$ antagonists) are a relatively new class of agents that inhibit the renin-angiotensin–aldosterone axis at the level of the angiotensin II receptor. These agents have a profile of hemodynamic effects similar to that of the converting enzyme inhibitors. Recent clinical trials have demonstrated a mortality benefit for $AT_1$ antagonists in patients with severe CHF (LV ejection fraction, <40%). Interestingly, this mortality benefit is additive with that of a concomitantly administered ACE inhibitor (CHARM trials).

## β-Adrenoceptor Antagonists

Much recent attention has been directed at the use of β-adrenoceptor antagonists in the treatment of patients with heart failure. Although the use of β-antagonists might seem counterintuitive, clinical trials have now established that these agents increase survival in heart failure patients. The benefits of β-antagonists in patients with heart failure have been variably attributed to β-antagonist mediated: (1) inhibition of renin release; (2) attenuation of the cytotoxic and signaling effects of elevated circulating catecholamines; and, more generally; (3) secondary prevention of acute coronary events. Thus, β-antagonists, like ACE inhibitors, may attenuate the adverse effects of neurohumoral regulators in patients with congestive heart failure. Furthermore, because β antagonists and ACE inhibitors act through distinct mechanisms, it is reasonable to coadminister these drugs to CHF patients.

### Vasodilators

Hydralazine is a direct-acting vasodilator that decreases systemic vascular resistance and, thereby, reduces afterload. The mechanism of action of hydralazine remains to be determined. The arterial vasodilation produced by hydralazine is particularly pronounced when the drug is administered intravenously. The clinical use of hydralazine has been limited by a number of factors, including the induction of reflex tachycardia during intravenous administration, the development of tachyphylaxis, and the occurrence of a drug-induced lupus syndrome during chronic administration. This agent has demonstrated a mortality benefit in CHF when coadministered with organic nitrates; the nitrate–hydralazine combination is typically reserved for patients who cannot tolerate therapy with an ACE inhibitor.

## INOTROPIC AGENTS

### Cardiac Glycosides

In cardiac myocytes, digitalis glycosides inhibit the sarcolemmal $Na^+/K^+$ ATPase. This action, in turn, increases intracellular sodium, activates the $Na^+$-$Ca^{2+}$ exchanger, and increases intracellular calcium, including the calcium stores in the sarcoplasmic reticulum. The increased calcium release upon myocyte stimulation potentiates interaction of the contractile proteins, resulting in increased myocardial contractility (leftward shift of the ESPVR). Although patients with congestive heart failure often experience relief of congestive symptoms upon treatment with the cardiac glycosides, these drugs have not been shown to decrease mortality in this disease.

### Sympathomimetic Amines

Dobutamine is the sympathomimetic amine used most commonly in the treatment of heart failure attributable to systolic dysfunction. This agent is a synthetic congener of epinephrine that stimulates $\beta_1$-receptors and, to a lesser extent, $\beta_2$-receptors and $\alpha_1$-receptors. At therapeutic infusion rates, the stimulation of $\beta_1$-receptors predominates, leading ultimately to an increase in the contractility of cardiac myocytes. Stimulation of peripheral $\beta_2$-receptors by dobutamine causes peripheral arterial vasodilation and a reduction in afterload. The combined effects of increased contractility and decreased afterload lead to improvement in overall cardiac performance.

### Phosphodiesterase Inhibitors

Such phosphodiesterase inhibitors as amrinone and milrinone inhibit the degradation of cAMP in cardiac myocytes and, thereby, increase intracellular calcium and enhance contractility. In the systemic vasculature, these agents cause dilation of both arteriolar resistance vessels and venous capacitance vessels, thereby decreasing afterload and preload. As a result of these aggregate effects, the phosphodiesterase inhibitors have been referred to as "ino-dilators." Despite these positive actions, both the phosphodiesterase inhibitors and the sympathomimetic amines are reserved for short-term treatment of patients with acute decompensation of heart failure. Indeed, long-term treatment with the phosphodiesterase inhibitors has been shown to *increase* mortality.

## COMBINATION THERAPY

The drugs described in this chapter offer a number of approaches to the pharmacotherapy of heart failure. Some agents, most notably ACE inhibitors and β-blockers, have demonstrated significant mortality benefit in randomized clinical trials and should probably be viewed as the new cornerstones of therapy. Others drugs, such as digoxin and diuretics, have been mainstays of symptomatic relief despite a lack of mortality benefit.

Use of combination therapies must be approached cautiously in CHF patients to avoid such side effects as hypotension, arrhythmias, electrolyte imbalances, and renal insufficiency. Nonetheless, it is typical for these patients to require multidrug regimens to optimize their functional status.

## ■ *Conclusion and Future Directions*

Hypertension, ischemic heart disease, and congestive heart failure are common cardiovascular diseases that typically occur in sequence. A number of therapeutic strategies have been developed to target the multiple cellular and molecular pathways that are dysfunctional in these diseases. All of these diseases possess complex pathophysiologies, however, that often cannot be adequately addressed with a single pharmacologic intervention. In many cases, therefore, these drugs are used in combination to achieve the desired therapeutic goal.

Two active areas of research that promise to provide new understanding of the pathophysiology of cardiovascular disease are genetic predispositions and neurohormonal pathways. For example, it has been hypothesized that the pathophysiology of essential hypertension will, in many cases, involve mutations or polymorphisms in the genes that code for angiotensinogen, renin, the angiotensin II receptor ($AT_1$), endothelin, the glucocorticoid receptor, the insulin receptor, endothelial nitric oxide synthase, and the epithelial $Na^+$ channel (ENaC). As the genetic determinants of cardiovascular regulation are clarified, it may be possible to identify high-risk patients prospectively and to develop targeted therapies that exert their therapeutic effects on the molecular and cellular mechanisms predicted to drive the disease in these patients.

In recent years, agents targeting neurohumoral pathways—such as ACE inhibitors and β-blockers—have become cornerstones of therapy for all cardiovascular disease. Large-scale clinical trials have consistently demonstrated mortality reduction for these agents, which exert their therapeutic effects by interrupting the neurohormonal systems that are activated in advanced heart failure and that may, in fact, drive progression of myocardial disease. Over the past two decades, the success of therapies that target circulatory regulation has led to a major shift in the focus of heart failure management, which now includes prevention of progressive cellular dysfunction as a primary goal. Current research efforts aim to identify and characterize new drug targets, including a host of signaling molecules that are abnormal in the failing heart. Elevated levels of inflammatory mediators such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and endothelin-1, and enzymes such as inducible nitric oxide synthase, collagenases, and matrix metalloproteinases, have all been reported to contribute in some way to the detrimental structural and functional changes that occur in the failing heart.

## *Suggested Readings*

### *Hypertension*

August P. Initial treatment of hypertension. N Engl J Med 2003;348:610–617. (*Overview of approaches to hypertension treatment*)

Chobanian AV, Bakris GL, Black HR, Cushman WC, et al. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 report. J Amer Med Assoc 2003;289: 2560–2571. (*Current guidelines for classifying and treating hypertension*)

Kaplan NM. Systemic hypertension: Therapy. In: Braunwald E, Zipes D, Libby P, eds. Heart Disease. 6th Ed. Philadelphia: WB Saunders Co, 2001:972–993. (*Overview of hypertension treatment*)

### *Ischemic Heart Disease*

Braunwald E, Antman EM, Beasley JW, Califf RM, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST elevation myocardial infarction. Summary article: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients with Unstable Angina). Circulation 2002;106:1893–1900. (*Current guidelines for evaluating and treating patients with unstable angina and non-ST-segment elevation MI*)

Cannon CP. New guidelines for the management of unstable angina and non-ST elevation myocardial infarction. Part I: Medical therapies. CardiologyRounds 2003;7(3):1–6. (Guidelines for evaluating and treating patients with unstable angina and non-ST-segment elevation MI)

Gupta A, Sabatine MS, Lilly LS. Ischemic heart disease. In: Lilly LS, ed. Pathophysiology of Heart Disease. 3rd Ed. Baltimore: Lippincott Williams & Wilkins, 2003:131–156. (*Mechanistic explanation of ischemic heart disease pathophysiology*)

Gupta A, Sabatine MS, O'Gara PT, Lilly LS. Acute coronary syndromes. In: Lilly LS, ed. Pathophysiology of Heart Disease. 3rd Ed. Baltimore: Lippincott Williams & Wilkins, 2003:157–184. (*Mechanistic explanation of acute coronary syndrome pathophysiology*)

### *Congestive Heart Failure*

Francis GS. Pathophysiology of chronic heart failure. Amer J Med 2001;110(7A):37S–46S. (*Reviews data emphasizing the primacy of neuroendocrine and cytokine activation in the progression of CHF, with discussion of new potential therapies*)

Jessup M, Brozena S. Medical Progress: Heart failure. N Engl J Med 2003;348:2007–2018. (*Reviews epidemiology and management of congestive heart failure*)

Opie LH, Gersh BJ. Drugs for the Heart. Philadelphia: WB Saunders Co, 2001. (*Chapters 4 (Diuretics), 5 (ACE inhibitors), and 6 (Digitalis and acute inotropes) summarize the rationale for use of these agents in treating CHF*)

# IV

# Principles of Endocrine Pharmacology

# 24

# Pharmacology of the Hypothalamus and Pituitary Gland

Ehrin J. Armstrong and Armen H. Tashjian, Jr.

**Introduction**
**Case**
**Hypothalamic and Pituitary Physiology**
  Relationship Between the Hypothalamus and Pituitary Gland
  Feedback Inhibition
**Physiology, Pathophysiology, and Pharmacology of Individual Axes**
  Anterior Pituitary Gland
    *Hypothalamic-Pituitary–Growth Hormone Axis*
    *Hypothalamic-Pituitary–Prolactin Axis*
    *Hypothalamic-Pituitary–Thyroid Axis*
    *Hypothalamic-Pituitary–Adrenal Axis*
    *Hypothalamic-Pituitary–Reproductive Axis*
  Posterior Pituitary Gland
    *Antidiuretic Hormone (ADH)*
    *Oxytocin*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

The hypothalamus and pituitary gland function cooperatively as master regulators of the endocrine system. Together, hormones secreted by the hypothalamus and pituitary gland control important homeostatic and metabolic functions, from reproduction to control of thyroid physiology. This chapter introduces the physiology and regulation of hypothalamic and pituitary hormones through a discussion of feedback regulation and the various axes of hormonal regulation. It then discusses the pharmacologic utility of hypothalamic and pituitary factors, with emphasis on the regulation of specific endocrine pathways. Three concepts are of special importance in this chapter: (1) hypothalamic control of pituitary hormone release; (2) negative feedback inhibition; and (3) endocrine axes. A thorough understanding of these pathways and their mechanisms provides a background for understanding all the chapters in this section.

## Case

Bobby has always been a small child. At school, he is sometimes picked on because of his short stature; kids occasionally taunt him as a "dwarf." When Bobby is in sixth grade, his pediatrician predicts that Bobby will be only about 4′6″ tall as an adult. Several tests are performed to seek an explanation for Bobby's small size. Laboratory results show low plasma growth hormone levels. Experimental diagnostic administration of synthetic growth-hormone releasing hormone increases Bobby's plasma growth hormone to near normal concentrations. Based on these findings, the pediatrician prescribes recombinant human growth hormone, which Bobby's mother injects three times per week. Bobby hates the needle, and the injections become such a struggle that the pediatrician tries prescribing a new nasal spray of hexarelin, an experimental growth hormone-releasing peptide analogue, which Bobby doesn't seem to mind. Two years later, Bobby's therapy is switched to a depot injection form of human growth hormone, so that he has to receive only one injection per month. At 16 years old, Bobby reaches a height of 5′10″, two inches taller than his dad.

## QUESTIONS

1. Based on the physician's tests, what can one conclude about the probable etiology of Bobby's early growth retardation?
2. Why did Bobby have to receive injections of growth hormone, rather than taking the hormone orally?
3. What advances allowed use of a nasal spray of hexarelin? How does the depot form of growth hormone act to decrease the frequency of injections?

# HYPOTHALAMIC AND PITUITARY PHYSIOLOGY

## RELATIONSHIP BETWEEN THE HYPOTHALAMUS AND PITUITARY GLAND

From a developmental perspective, the pituitary gland consists of two closely associated organs. The **anterior pituitary** (adenohypophysis) is derived from ectodermal tissue. The **posterior pituitary** (neurohypophysis) is a neural structure derived from the ventral surface of the diencephalon. The prefixes adeno- and neuro- denote the buccal ectodermal and neural ectodermal origin of the anterior and posterior pituitary gland components, respectively. An intermediate lobe also exists in most mammals but is vestigial in humans.

Although the anterior and posterior pituitary glands derive from different embryologic origins, the hypothalamus controls the activity of both lobes. The mode of connection between hypothalamus and pituitary gland is one of the most important points of interaction between the nervous and endocrine systems. The hypothalamus acts as a neuroendocrine transducer by integrating neural signals from the brain and converting those signals into chemical messages that regulate the secretion of pituitary hormones. The pituitary hormones in turn alter the activities of peripheral endocrine organs.

Hypothalamic control of the anterior pituitary gland occurs via hypothalamic secretion of hormones into the **hypothalamic–pituitary portal vascular system** (Fig. 24-1). The initial capillary bed of this portal system is formed from branches of the superior hypophyseal arteries that fan around the hypothalamus. Endothelial fenestrations in this capillary bed allow hypothalamic factors to be released into the bloodstream. These capillaries then coalesce into short veins that flow to the anterior pituitary gland. Upon arriving at the anterior pituitary, the veins branch into a second capillary bed and bathe the cells of the anterior pituitary gland with hormones secreted by the hypothalamus.

A direct neural connection exists between the hypothalamus and posterior pituitary gland. Neurons in the hypothalamus synthesize hormones, destined for storage in the posterior pituitary gland, in cell bodies of the supraoptic and paraventricular nuclei. These hormones are then transported down axons to terminals in the posterior pituitary gland, where they are stored in neuronal terminals until a release stimulus occurs. The posterior pituitary gland can, therefore, be thought of as an extension of the hypothalamus. As with the anterior pituitary gland, the endothelial cells surrounding the posterior pituitary gland are fenestrated; this facilitates release of hormones into the systemic circulation.

The anterior pituitary gland is a heterogeneous collection of numerous cell types, each of which has the capacity to respond to specific stimuli and consequently release specific hormones. There are a number of hypothalamic releasing or inhibiting factors, each of which alters the hormone secretion pattern of one or more anterior pituitary gland cell types (Table 24-1). Releasing factors also modify other cellular processes in the anterior pituitary gland, including hormone synthesis and pituitary cell growth. Interestingly, *the relationship between hypothalamic-releasing factors and pituitary gland hormones is not always 1:1, nor is the interaction always stimulatory.* Somatostatin, for example, primarily inhibits the release of growth hormone, but it can also inhibit release of thyroid stimulating hormone (TSH) and prolactin. Conversely, thyrotropin-releasing hormone primarily stimulates the release of TSH, but it can also cause release of prolactin. These overlapping activities between releasing factors and the antagonistic behavior of some release-inhibiting hormones provide a mechanism for the precise regulation of secretory pathways.

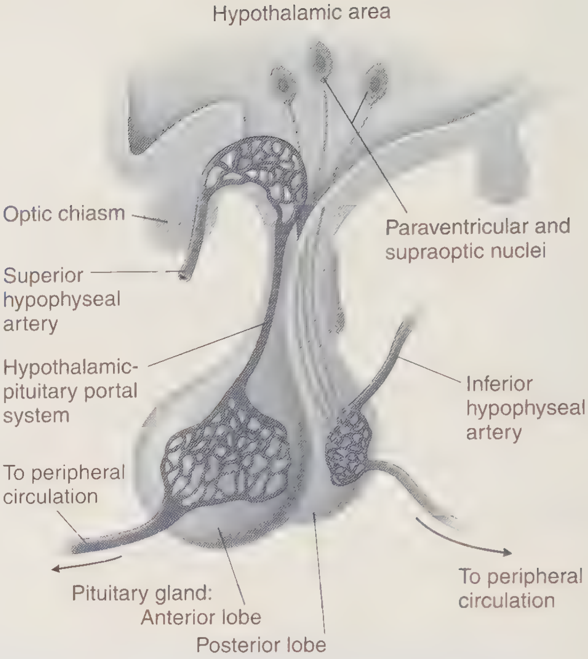

Hypothalamic area
Optic chiasm
Superior hypophyseal artery
Hypothalamic-pituitary portal system
To peripheral circulation
Paraventricular and supraoptic nuclei
Inferior hypophyseal artery
To peripheral circulation
Pituitary gland:
Anterior lobe
Posterior lobe

**Figure 24-1. The Hypothalamic–Pituitary Portal System.** Neurons in the hypothalamus release regulatory factors that are carried by the hypothalamic–pituitary portal system to the anterior pituitary gland, where they control the release of the anterior pituitary hormones. The posterior pituitary hormones are synthesized in cell bodies of the supraoptic and paraventricular neurons in the hypothalamus, and then transported down axonal pathways to terminals in the posterior pituitary gland. These hormones are stored in the posterior pituitary gland, from which they are released into the peripheral circulation. Note the separate vascular supplies to the anterior and posterior lobes of the pituitary gland.

*With the exception of dopamine, all known hypothalamic-releasing factors are peptides. The anterior pituitary gland hormones are proteins and glycoproteins.* Anterior pituitary gland hormones fall into three groups. Somatotropic hormones, consisting of **growth hormone** (GH) and **prolactin,** are 191 and 198 amino acids long, respectively, and exist as monomeric proteins. Glycoprotein hormones, consisting of **lutenizing hormone** (LH), **follicle-stimulating hormone** (FSH) and **thyroid-stimulating hormone** (TSH), are heterodimeric proteins with carbohydrates attached to certain residues. **Adrenocorticotropin** (ACTH) belongs to a separate class, as it is processed from a larger precursor protein. Of importance, intact peptides and proteins are not absorbed across the intestinal lumen; local proteases digest them into their constituent amino acids. For this reason, therapeutic replacement of a hypothalamic or pituitary hormone, as in Bobby's case, must be accomplished by a nonoral route or by an orally available nonpeptide analogue of the natural hormone.

**TABLE 24-1** Anterior Pituitary Gland Cell Types, Hypothalamic Control Factors, and Hormonal Targets

| ANTERIOR PITUITARY GLAND CELL TYPE | STIMULATORY HYPOTHALAMIC FACTORS | INHIBITORY HYPOTHALAMIC FACTORS | PITUITARY HORMONES RELEASED | MAJOR TARGET ORGAN | TARGET GLAND HORMONES |
|---|---|---|---|---|---|
| Somatotroph | GHRH, Ghrelin | Somatostatin | GH | Liver | Insulin-like growth factors |
| Lactotroph | Possibly TRH | Dopamine, Somatostatin | Prolactin | Mammary gland | None |
| Gonadotroph | GnRH | None known | LH and FSH | Gonads | Estrogen, progesterone, and testosterone |
| Thyrotroph | TRH | Somatostatin | TSH | Thyroid gland | Thyroxine and triiodothyronine |
| Corticotroph | CRH | None known | ACTH | Adrenal cortex | Cortisol, adrenal androgens |

Each anterior pituitary gland cell type responds to multiple hypothalamic stimulatory and inhibitory factors. Integration of these signals determines the relative extent of hormone release by the anterior pituitary gland. Each hormone has one or more specific target organs, which are, in turn, stimulated to release their own hormones. These target hormones cause feedback inhibition at the hypothalamus and anterior pituitary gland.

The anterior pituitary gland response to a hypothalamic factor is signaled through binding of the hypothalamic factor to specific G protein-coupled receptors present on the plasma membrane of the appropriate anterior pituitary cell type. Most of these receptors alter the levels of intracellular cAMP or $IP_3$ and calcium (see Chapter 1, Drug–Receptor Interactions). Knowledge of the molecular details of receptor signaling provides a basis for hypothalamic factor action. For example, **growth hormone-releasing hormone** (GHRH) binding to receptors on somatotroph cells increases intracellular cAMP and $Ca^{2+}$ levels; whereas, somatostatin has been shown to decrease intracellular levels of cAMP and $Ca^{2+}$ in somatotrophs. This provides a biochemical explanation for the opposing activities of GHRH and somatostatin on somatotroph release of growth hormone.

The timing and pattern of hypothalamic factor release are important determinants of anterior pituitary cell response. *Most hypothalamic-releasing factors are secreted in a cyclical or pulsatile, rather than continuous, manner.* For example, the hypothalamus releases gonadotropin-releasing hormone (GnRH) with a periodicity of a few hours. The frequency and magnitude of GnRH release determines the extent of pituitary gonadotropin release as well as the ratio of LH secretion to FSH secretion. Continuous administration of GnRH suppresses rather than stimulates pituitary gonadotroph activity. The different pharmacologic effects of GnRH, depending on the frequency and pattern of administration, have important clinical consequences, as discussed below. Although not studied in as much detail, the majority of hypothalamic-releasing factors are thought to be released in a pulsatile manner.

## FEEDBACK INHIBITION

End-product inhibition tightly controls hypothalamic and pituitary gland hormone release. For each hypothalamic-pituitary–target organ system, an integrated picture of how each set of hormones affects a given physiologic system can be constructed. Each pathway, including a hypothalamic factor(s), its pituitary gland target cell type, and the ultimate target gland(s), is referred to as an **endocrine axis;** the term "axis" is used to connote one of multiple homeostatic systems that the hypothalamus and pituitary gland control. A simplified model consists of five endocrine axes, with a single type of anterior pituitary gland cell at the center of each axis (Table 24-1).

Each axis regulates an important aspect of endocrine homeostasis and is, therefore, subject to close regulation. Feedback inhibition is usually discussed in terms of loops, because the regulatory connection between a given hormone and its target creates a "loop" that alters the subsequent extent of hormone release. These loops, depending on the hormone and its target organ, are commonly referred to as long loops, short loops, and ultrashort loops (Fig. 24-2). The terminology is imprecise, primarily indicating the relative distance a hormone must travel to the regulated organ. The long loop involves feedback regulation of a systemic hormone on the hypothalamus or pituitary gland. The short loop consists of pituitary hormone acting on the hypothalamus to alter release of hypothalamic factors. Finally, ultrashort-loop feedback involves hypothalamic or pituitary hormones directly regulating the cells that secreted the hormone; ultrashort feedback is synonymous with autocrine or paracrine signaling.

Just as regulatory loops are referred to based on a hormone's relationship to its target organ, many endocrine diseases are described based on whether the disease etiology is a disorder of the hypothalamus, pituitary gland, or target organ. *The disease is referred to as primary, secondary, or tertiary, depending on whether the underlying abnormality is in the target organ, pituitary gland, or hypothalamus, respectively.* Therefore, a primary endocrine disorder is caused by target tissue pathology, a secondary disorder reflects pituitary disease, and a tertiary endocrine disorder results from hypothalamic pathology. Whether the underlying disease cause is primary,

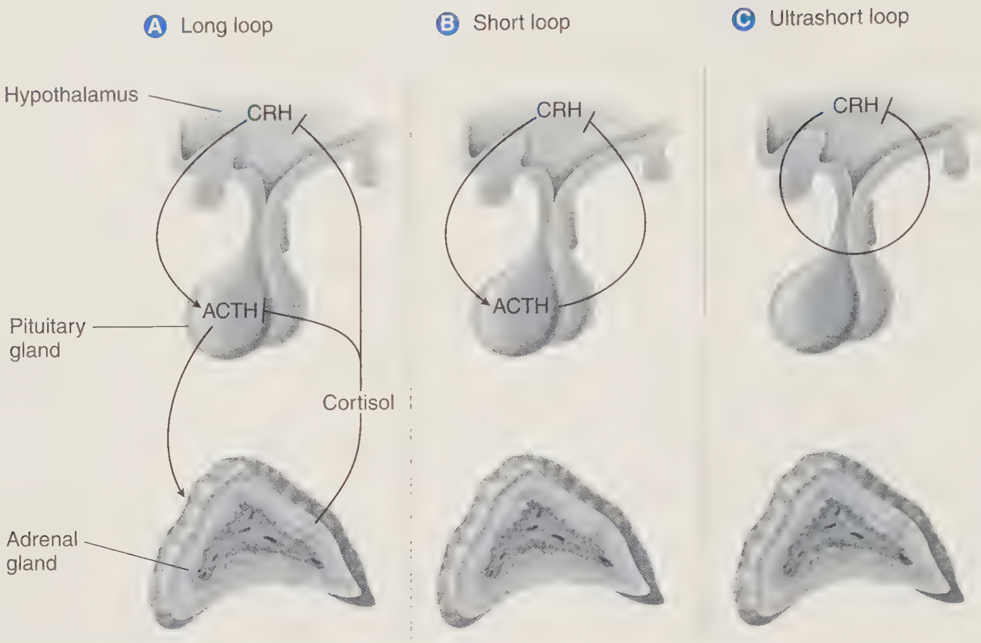


**Figure 24-2. Hypothalamic-Pituitary–Target Organ Feedback.** There are three general mechanisms of feedback regulation within the hypothalamic-pituitary–target organ axes, referred to as long-loop, short-loop, and ultrashort-loop feedback. Here, the hypothalamic-pituitary–adrenal axis is used to illustrate these concepts. **A,** In long-loop feedback, the target organ produces a hormone such as cortisol that, in addition to its physiologic actions on target tissues, inhibits anterior pituitary gland release of adrenocorticotropic hormone (ACTH) and hypothalamic release of corticotropin releasing hormone (CRH). **B,** In short-loop feedback, a hormone produced by the anterior pituitary gland, such as ACTH, inhibits hypothalamic release of its own releasing hormone, CRH. **C,** In ultrashort-loop feedback, the hormone produced by the hypothalamus negatively regulates itself; for example, CRH inhibits further CRH release.

secondary, or tertiary can have important consequences for disease diagnosis and treatment, as discussed below.

# PHYSIOLOGY, PATHOPHYSIOLOGY, AND PHARMACOLOGY OF INDIVIDUAL AXES

## ANTERIOR PITUITARY GLAND

### Hypothalamic-Pituitary–Growth Hormone Axis

The hypothalamic-pituitary–growth hormone axis regulates a number of general processes promoting growth. Growth hormone is first expressed at high concentrations during puberty; growth hormone secretion during this time is pulsatile, with the largest pulses usually occurring at night. Most of the anabolic effects of growth hormone are mediated by insulin-like growth factors, especially **insulin-like growth factor 1** (IGF-1), a hormone released by hepatocytes in response to stimulation by growth hormone.

Growth hormone secretion is enhanced by growth hormone–releasing hormone (GHRH) and inhibited by somatostatin. Recently, a new endogenous growth hormone-releasing peptide, known as **ghrelin,** has been isolated. Ghrelin promotes somatotroph secretion of growth hormone via a receptor that is different from the GHRH receptor. Ghrelin and GHRH seem to act synergistically on growth hormone release. In addition, ghrelin seems to be an important regulator of appetite. Nonpeptide orally active ghrelin mimetics are currently under clinical investigation.

Failure to secrete growth hormone or to enhance IGF-1 secretion during puberty results in growth retardation (Fig. 24-3). Growth hormone deficiency most commonly results from defective hypothalamic release of GHRH (tertiary disease) or pituitary insufficiency (secondary disease). Importantly, however, failure of IGF-1 secretion in response to growth hormone (Laron dwarfism) is one etiology of short stature not amenable to growth hormone therapy. **Sermorelin** (synthetic GHRH) can be administered parenterally to determine the disease etiology. If a patient possesses defective hypothalamic release of GHRH but normally functioning anterior pituitary gland somatotrophs, administration of exogenous GHRH results in increased GH release. This result was seen in the introductory case where, despite low baseline levels of growth hormone, injection of GHRH increased Bobby's plasma GH concentration. Because Bobby's GH levels increased after GHRH administration, he likely had tertiary growth hormone deficiency. *If defective pituitary gland function is the etiology of a patient's growth hormone deficiency, administration of sermorelin will not alter plasma growth hormone levels.*

Most cases of growth hormone-dependent growth retardation are treated with replacement **recombinant human growth hormone.** Typical dosage schedules involve subcutaneous or intramuscular injection three times per week. To overcome this inconvenience, alternative delivery methods for growth hormone have recently been developed, including a slow-release depot injection of growth hormone that requires injection only once per month. This depot formulation works by incorporating GH into a polymer matrix that degrades slowly at the injection site to release GH (see Chapter 50, Drug Delivery Mechanisms).

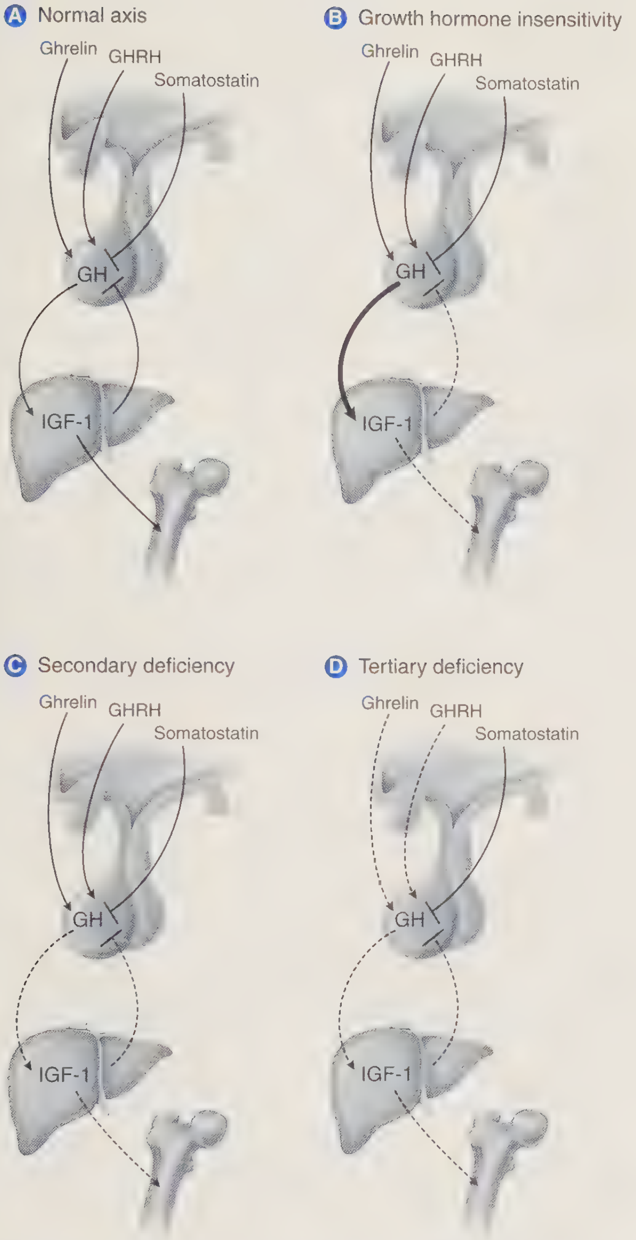


**Figure 24-3. Hypothalamic-Pituitary–Growth Hormone Axis in Health and Disease.** **A,** In the normal hypothalamic-pituitary–growth hormone axis, hypothalamic secretion of growth hormone-releasing hormone (GHRH) or ghrelin stimulates release of growth hormone (GH), while somatostatin inhibits release of GH. Secreted growth hormone then stimulates the liver to synthesize and secrete insulin-like growth factor I (IGF-1), which promotes bone growth. IGF-1 also inhibits GH release from the anterior pituitary gland. **B,** In growth hormone insensitivity, the anterior pituitary gland secretes growth hormone, but the liver is unresponsive to stimulation by growth hormone. As a result, IGF-1 is not secreted (indicated by dashed lines). The decreased feedback inhibition of GH release results in higher plasma levels of GH (thick line). **C,** In secondary deficiency, the pathology lies in an unresponsive anterior pituitary gland, which does not secrete growth hormone. Because GH levels are low, the liver is not stimulated to produce IGF-1. **D,** In tertiary deficiency, the hypothalamus does not secrete GHRH (dashed line); the role of ghrelin in this condition is unknown. Lack of GHRH results in lack of stimulation of GH secretion by the anterior pituitary gland and, therefore, diminished production of IGF-1.

Cases where growth hormone deficiency is caused by a tertiary, hypothalamic deficit of GHRH can be treated with sermorelin (GHRH), but it is believed that this agent may have less efficacy than GH therapy. For this reason, GH is the preferred agent for treatment of short stature. New treatments being investigated as substitutes for growth hormone injection include **hexarelin,** a 6-amino acid peptide thought to stimulate pituitary gland GH secretion via a receptor that is distinct from the GHRH receptor (so-called GHRH secretagogues). The potential advantages of hexarelin include intranasal administration and a longer half-life relative to native GHRH. Nonpeptide GHRH and orally active secretagogues are also currently in late-stage clinical investigation. Because Bobby presumably had a tertiary growth hormone deficiency, nasal hexarelin was a possible therapy for his short stature.

Growth hormone excess usually results from a somatotroph adenoma. This entity has two differing disease presentations, depending on whether the growth hormone excess occurs before or after closure of the bone epiphyses. Gigantism will occur if growth hormone is secreted at high levels before closure of the epiphyses, because increased IGF-1 levels will promote excessive longitudinal bone growth. After the epiphyses close, increases in growth hormone result in **acromegaly.** This condition occurs because IGF-1, although it can no longer stimulate long bone growth, can still promote growth of deep organs and cartilaginous tissue, resulting in such physical alterations as increased finger thickness, large facial structures, and hepatomegaly.

Standard treatment for a somatotroph adenoma is transsphenoidal surgical removal of the tumor, but medical treatment with injected **octreotide,** a synthetic long-acting peptide analogue of somatostatin, can also be effective for small tumors or to decrease tumor size before surgery. Somatostatin itself is rarely used clinically because it has a half-life of only a few minutes. Because somatostatin and its analogues affect numerous secretory processes, octreotide can be used for several indications, including treatment of esophageal varices and certain hormone-secreting tumors. The mechanism by which octreotide ameliorites esophageal varices is unknown, but is thought to involve selective vasoconstriction of arteriolar sphincters in the splanchnic circulation. Systemic administration of octreotide can lead to side effects, including nausea and decreased gastrointestinal motility. As with growth hormone, prolonged release formulations of octreotide are currently under study.

## Hypothalamic-Pituitary–Prolactin Axis

Lactotrophs of the anterior pituitary gland produce and secrete prolactin. Their activity is decreased in response to hypothalamic secretion of **dopamine. Thyrotropin-releasing hormone** (TRH) can enhance prolactin release. Unlike other cells of the anterior pituitary gland, lactotrophs are under tonic inhibition by the hypothalamus, presumably mediated by hypothalamic release of dopamine. *Therefore, a disease condition interrupting the hypothalamic–pituitary portal system results in decreased secretion of most anterior pituitary gland hormones but causes increased prolactin release.* In patients taking phenothiazine antipsychotics (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission), elevations of prolactin are frequently observed because these agents are dopamine receptor antagonists. In addition, prolactin does not stimulate secretion of hormones in its target, the mammary gland. It is, therefore, not regulated by a negative feedback system.

The physiologic actions of prolactin involve regulation of mammary gland development and milk protein biosynthesis and secretion. Prolactin levels are normally low in men and nonpregnant women. Increased estrogen levels during pregnancy stimulate lactotroph cells of the anterior pituitary gland to secrete increasing quantities of prolactin. During pregnancy, however, estrogen antagonizes prolactin action in the breast; this prevents lactation until after parturition occurs. Suckling provides a powerful stimulus for prolactin release; prolactin levels increase as much as 100-fold within 30 minutes after the initiation of breast feeding. If a mother does not breast feed, prolactin levels decrease over the course of several weeks.

Interestingly, increased prolactin levels suppress estrogen synthesis, both by antagonizing hypothalamic release of GnRH and by decreasing gonadotroph sensitivity to GnRH. This results in decreased LH and FSH release and hence, decreased end-organ stimulation of the hypothalamic-pituitary–reproductive axis. This may be a physiologic mechanism to suppress ovulation while a mother is still breast feeding. *Chronically high secretion of prolactin attributable to a prolactinoma also suppresses the hypothalamic-pituitary–reproductive axis.* For this reason, prolactinomas are a common cause of infertility, especially in women.

Administration of **bromocriptine,** a synthetic dopamine receptor agonist, is a proved medical therapy for small prolactinomas (microadenomas) because bromocriptine inhibits lactotroph cell growth. Bromocriptine is orally bioavailable. As with octreotide, many of the side effects of bromocriptine therapy result from systemic actions of the drug. The side effects include nausea and vomiting, presumably because the area postrema in the medulla, which stimulates nausea, possesses receptors for dopamine. Other members of this class of dopamine receptor agonists include **pergolide, quinoglide,** and **cabergoline.** All seem to possess equal therapeutic efficacy. Cabergoline, because it differs structurally from other dopamine receptor agonists, may cause less nausea and vomiting than the other agents.

## Hypothalamic-Pituitary–Thyroid Axis

The hypothalamus secretes **thyrotropin-releasing hormone** (TRH), which stimulates thyrotrophs to produce and secrete **thyroid-stimulating hormone** (TSH). TSH in turn promotes biosynthesis and secretion of thyroid hormone by the thyroid gland. Thyroid hormone regulates overall body energy homeostasis. Thyroid hormone negatively controls hypothalamic and pituitary release of TRH and TSH, respectively.

Because thyroid hormone replacement is an effective therapy for hypothyroidism, TRH and TSH are used mainly for diagnosis of disease etiology. If hypothyroidism is caused by an unresponsive thyroid gland, TSH levels will be high because of decreased negative feedback from thyroid hormone. If the anterior pituitary gland is unable to produce TSH in response to TRH, then pharmacologic administration of TSH should result in production and release of thyroid hormone. Finally, if the disorder is hypothalamic in origin (a tertiary endocrine disorder), addition of either exogenous TRH or exogenous TSH will stimulate increased plasma levels of thyroid hormone. Other aspects of thyroid gland pharmacology are discussed in Chapter 25, Pharmacology of the Thyroid Gland.

## Hypothalamic-Pituitary–Adrenal Axis

Neurons from the paraventricular nucleus of the hypothalamus synthesize and secrete **corticotropin-releasing hormone** (CRH). After transport via the hypothalamic–pituitary portal system, CRH binds to cell surface receptors present on corticotrophs of the anterior pituitary gland. CRH binding stimulates corticotrophs to synthesize and release adrenocorticotropin-releasing hormone (ACTH). ACTH is synthesized as part of proopiomelanocortin (POMC), a precursor polypeptide that is cleaved into multiple effector molecules. In addition to ACTH, cleavage of POMC yields melanocyte stimulating hormone (MSH), lipotropin, and β-endorphin. MSH has effects on skin pigmentation, feeding behavior, and obesity. Because of the structural similarities between ACTH and MSH, high concentrations of ACTH can bind to and activate MSH receptors. This becomes important in primary hypoadrenalism, where increased ACTH levels result in enhanced skin pigmentation. Once secreted, ACTH binds to ACTH receptors present on cells of the adrenal cortex, especially the zona fasciculata and zona reticularis of the cortex. ACTH stimulates the synthesis of adrenocortical steroid hormones, which include androgens, glucocorticoids, and mineralocorticoids. ACTH also has a trophic effect on the adrenal cortex; excessive ACTH secretion causes adrenal hyperplasia. The glucocorticoid cortisol is the main feedback inhibitor of pituitary ACTH release.

CRH is employed clinically to determine whether excessive cortisol secretion results from a pituitary adenoma or adrenal tumor (ectopic or primary) (Fig. 24-4). If the hypercortisolism derives from a pituitary adenoma, administration of CRH will usually increase blood ACTH and cortisol levels. This response is not seen in the case of an ectopic tumor, which will secrete ACTH at a constant autonomous level. Likewise, ACTH levels will not increase after administration of CRH if a patient has a primary adrenal tumor, because the excess cortisol secretion suppresses the pituitary ACTH response to CRH.

A synthetic form of ACTH, known as **cosyntropin,** can be used to diagnose suspected cases of adrenal insufficiency; administration of cosyntropin to a patient with adrenal insufficiency will fail to increase plasma cortisol concentration. Conditions requiring addition of glucocorticoids are usually treated with synthetic analogues of cortisol rather than ACTH because using the target hormone generally allows for better dosage control. Cortisol physiology and pharmacology are discussed in Chapter 26, Pharmacology of the Adrenal Cortex.

## Hypothalamic-Pituitary–Reproductive Axis

Gonadotrophs are unique among anterior pituitary gland cells because they secrete two hormones: **luteinizing hormone** (LH) and **follicle-stimulating hormone** (FSH); together, these hormones are referred to as gonadotropins. LH and FSH are both heterodimers composed of α and β subunits. LH and FSH share the same α subunit, but possess different β subunits. Gonadotrophs regulate the secretion of FSH and LH independently. This axis is diagrammed in Figure 24-5.

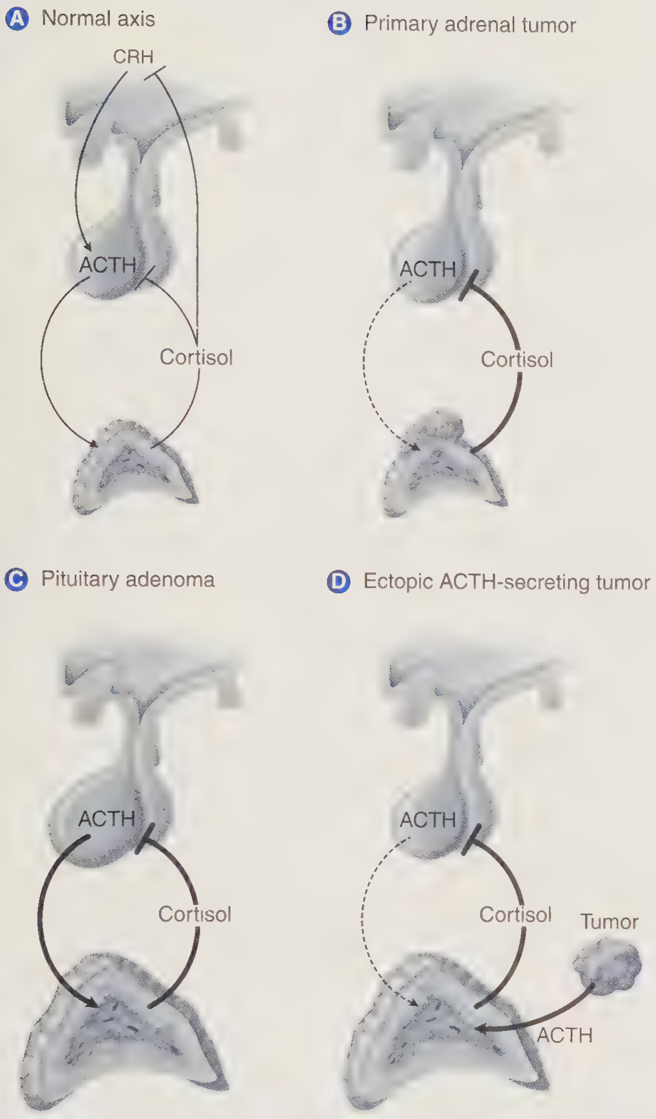


**Figure 24-4. Use of CRH to Determine the Cause of Excess Cortisol Secretion.** **A,** In the normal hypothalamic-pituitary–adrenal axis, hypothalamic secretion of corticotropin releasing hormone (CRH) stimulates release of adrenocorticotropic hormone (ACTH). ACTH, in turn, stimulates synthesis and secretion of cortisol by the adrenal cortex. Cortisol then inhibits further release of CRH and ACTH. **B,** A primary adrenal tumor autonomously produces cortisol (thick line), independent of regulation by ACTH. Administration of CRH will not increase ACTH and cortisol levels because the excessive cortisol produced by the tumor suppresses anterior pituitary gland responsiveness to CRH (dashed line). **C,** An adenoma (tumor) in the anterior pituitary gland autonomously secretes excessive levels of ACTH (thick line), which stimulate the adrenal gland to produce increased levels of cortisol (thick line). Although ACTH secretion by the tumor may not be sensitive to feedback inhibition by cortisol, the pituitary adenoma may be sensitive to CRH administration. In this case, administration of CRH stimulates an acute rise in ACTH and cortisol levels. **D,** An ectopic ACTH-producing tumor (such as a small cell carcinoma of the lung) also stimulates the adrenal gland to produce increased levels of cortisol. In this case, however, pituitary gland ACTH production is suppressed by the increased cortisol, and administration of CRH will not increase ACTH and cortisol levels. This example illustrates the clinical use of ACTH levels, cortisol levels, and the effect of CRH administration on ACTH and cortisol levels to determine the etiology of excess cortisol secretion.

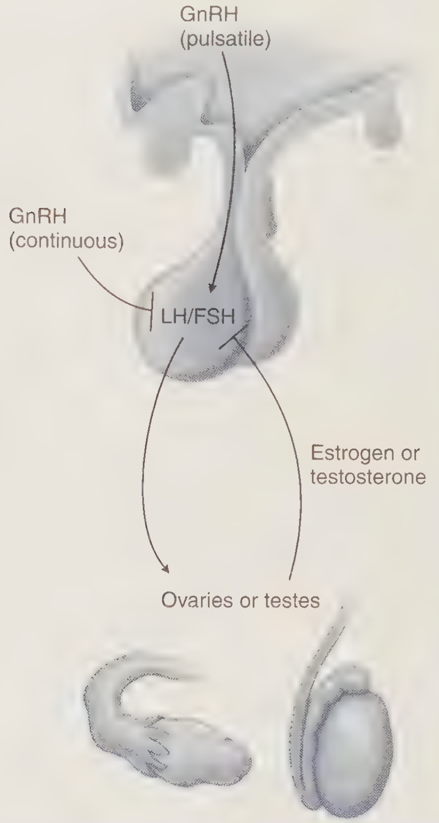


**Figure 24-5. Effects of GnRH on the Hypothalamic-Pituitary–Reproductive Axis.** Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus in a pulsatile fashion, stimulating gonadotroph cells of the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH stimulate the ovaries or testes to produce the sex hormones estrogen or testosterone, respectively, which inhibit further release of LH and FSH. Exogenous pulsatile GnRH is used to induce ovulation in women with infertility of hypothalamic origin. Conversely, continuous administration of GnRH suppresses gonadotroph response to endogenous GnRH and, thereby, causes decreased production of sex hormones. Analogues of GnRH with increased metabolic stability and prolonged half-lives take advantage of this effect, and are used to suppress sex hormone production in such clinical conditions as precocious puberty and prostate cancer.

Once secreted, gonadotropins control hormone production by the gonads, promoting the synthesis of androgens and estrogens. Gonadotrophs are then feedback-inhibited by testosterone and estrogen. The relationship of estrogen to the anterior pituitary gland is complex and consists of inhibition and excitation, depending on the rate of change and absolute concentrations of estrogen as well as the stage of the menstrual cycle. **Inhibin** and **activin** are two hormones secreted by the ovary that seem to have inhibitory and releasing effects, respectively, on FSH but not LH secretion. Endocrine control of the reproductive process is discussed in greater detail in Chapter 27, Pharmacology of Reproduction.

*Peptide GnRH analogues with short half-lives can be administered in a pulsatile fashion to stimulate patterned gonadotropin release, while analogues with longer half-lives are used to suppress production of sex hormones by desensitizing the pituitary gland to the stimulating activity of the releasing* *factor* (Fig. 24-5). The main pharmacologic difference among currently approved GnRH agonists is the method of administration. **Leuprolide** and **histrelin** are injected once daily; **nafarelin** is a nasal spray; **goserelin** is a depot injection administered once per month. Osmotic pump implants (see Chapter 50) are also available that deliver leuprolide acetate at a controlled rate for up to 12 months. Long-acting agonists are utilized therapeutically for treatment of several gonadotropin-dependent conditions, including endometriosis, uterine fibroids, precocious puberty, and androgen-dependent prostate cancer. Their main drawback is that gonadotroph suppression does not occur immediately; there is instead a transient (several days) increase in sex hormone levels, followed by a lasting suppression of hormone synthesis and secretion.

FSH is used clinically to stimulate ovulation for in vitro fertilization. Two formulations are available. **Urofollitropin** is purified FSH isolated from urine of postmenopausal women, whereas, **follitropin** is a recombinant DNA form of FSH. Both agents effectively stimulate ovulation but may cause **ovarian hyperstimulation syndrome.** It has recently been shown that a rare form of ovarian hyperstimulation syndrome occurring during pregnancy (familial gestational ovarian hyperstimulation syndrome) is caused by an inherited mutation in the FSH receptor. This mutation allows human chorionic gonadotropin (hCG), a hormone present in high concentrations during the early stages of pregnancy, to activate the FSH receptor. The resulting overstimulation of the FSH receptor is thought to cause the follicular enlargement and other sequelae characteristic of ovarian hyperstimulation syndrome. Whether similar mutations in the FSH receptor could be responsible for cases of iatrogenic ovarian hyperstimulation syndrome is an area of active investigation.

In contrast to the clinical success of GnRH agonists and FSH, development of an effective GnRH antagonist has been hampered by the side effect of excessive histamine release and anaphylaxis. Newer formulations under development seem to have fewer side effects, and several compounds are in late-stage clinical trials.

## POSTERIOR PITUITARY GLAND

In comparison to the numerous hormones of the anterior pituitary gland, the posterior lobe of the pituitary gland (neurohypophysis) secretes only two hormones, antidiuretic hormone (ADH) and oxytocin. ADH is an important regulator of plasma volume and osmolarity, whereas, oxytocin has physiologic effects on uterine contraction and lactation.

### Antidiuretic Hormone (ADH)

ADH is a peptide hormone produced by magnocellular cells of the hypothalamus. Cells of this region possess osmoreceptors that are able to sense increases in extracellular osmolarity. Increased osmolarity stimulates ADH secretion from nerve terminals in the posterior pituitary gland. ADH binds to two types of receptors, $V_1$ and $V_2$. $V_1$ receptors, present in systemic arterioles, mediate vasoconstriction. This property gives ADH its alternative name, vasopressin. $V_2$ receptors, present in the nephron, stimulate water channels to increase water reabsorption in the collecting duct, as discussed in Chapter 19, Pharmacology of Volume Regulation. These two actions of ADH combine to maintain vascular tone by: (1) increasing blood pressure; and (2) increasing water reabsorption.

Disruption of ADH homeostasis results in two important pathophysiologies. Excessive secretion of ADH causes the **syndrome of inappropriate ADH** (SIADH); deficient secretion of ADH, or decreased response to ADH causes diabetes insipidus. In SIADH, ADH secretion occurs irrespective of plasma volume or osmolality. One of the most common causes of SIADH is inappropriate secretion by a so-called small (oat) cell carcinoma of the lung, which can produce ADH ectopically. This results in persistent stimulation of $V_1$ and $V_2$ receptors, resulting in hypertension and excessive fluid retention. The inappropriate fluid retention can result in significant edema and low extracellular sodium concentration. If the source of excess ADH cannot be removed, the only effective therapy for SIADH is restriction of fluid intake or administration of hypertonic saline. No pharmacologic agents exist that are able to antagonize ADH action specifically; development of such a drug could be a potentially valuable therapy for SIADH. Currently, **demeclocycline** (a tetracycline antibiotic: see Chapter 33, Pharmacology of Transcription and Translation) and **lithium** are the only pharmacologic treatments effective for SIADH. Their mechanisms of action are not well understood, however, and use of these agents is limited by their nonspecific actions and potential for side effects. For example, lithium has a narrow therapeutic index and may cause irreversible renal damage (see Chapter 12, Pharmacology of Affective Disorders).

**Diabetes insipidus** is characterized by symptoms similar to diabetes mellitus, including thirst, polydipsia, and polyuria. Diabetes insipidus is a disorder of either vasopressin secretion or response, whereas, diabetes mellitus results in excessive plasma glucose because of either deficient insulin production or target tissue insensitivity to insulin. The etiologies of diabetes mellitus and diabetes insipidus are unrelated. Pathologic disturbances that inhibit the ability of ADH to stimulate water reabsorption at the nephron can result in diabetes insipidus. A distinction is made between two types of diabetes insipidus. **Neurogenic diabetes insipidus** results from an inability of hypothalamic neurons to synthesize or secrete ADH (Fig. 24-6). **Nephrogenic diabetes insipidus** results from an inability of the nephron to respond to ADH. This is usually caused by a mutation in the $V_2$ receptor, so that ADH is no longer able to bind or stimulate $V_2$ receptor signaling. Neurogenic diabetes insipidus can be treated with intranasal **desmopressin,** an ADH analogue that preferentially stimulates $V_2$ receptors. This has the advantage of little cross reactivity with $V_1$ receptors, thus not increasing blood pressure that might occur with ADH administration. Overdosage with desmopressin can result in hyponatremia, caused by excessive water reabsorption by the nephron. No specific pharmacologic treatment currently exists for nephrogenic diabetes insipidus; patients are usually treated by restriction of fluid intake or given diuretics to inhibit excessive dilution of the urine. A hypothetical pharmacologic therapy for nephrogenic diabetes insipidus might consist of a compound that directly stimulates expression of nephron collecting duct water channels, thus bypassing the nonfunctional $V_2$ receptor.

### Oxytocin

Oxytocin is a peptide hormone produced by paraventricular cells of the hypothalamus. Many of the known physiologic

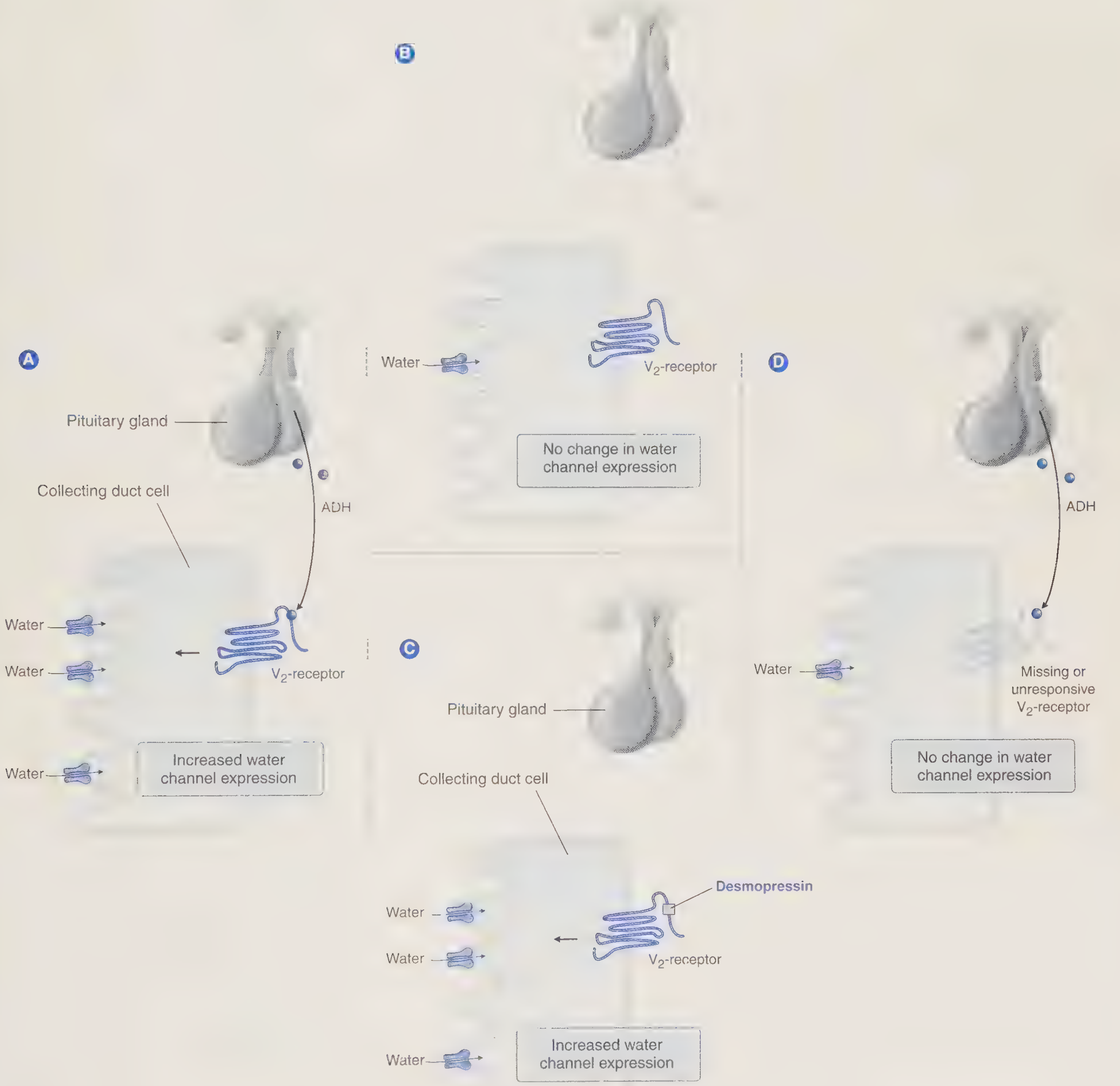

B
A
Pituitary gland
Collecting duct cell
ADH
Water
Water
Water
$V_2$-receptor
Increased water channel expression
Water
$V_2$-receptor
No change in water channel expression
D
ADH
Water
Missing or unresponsive $V_2$-receptor
No change in water channel expression
C
Pituitary gland
Collecting duct cell
Desmopressin
Water
Water
Water
$V_2$-receptor
Increased water channel expression

**Figure 24-6. Comparison of Neurogenic and Nephrogenic Diabetes Insipidus.** **A,** Antidiuretic hormone (ADH), released by nerve terminals in the posterior pituitary gland, stimulates $V_2$ receptors on renal collecting duct cells and, thereby, increases expression of water channels in the apical membrane of these cells. Increased water channel expression increases the water flux through the cell, and the increased water reabsorption helps to maintain extracellular fluid volume. **B,** In neurogenic diabetes insipidus, the posterior pituitary gland is unable to secrete ADH. Consequently, there is no stimulation of renal $V_2$ receptors by ADH, and the collecting duct cells are unable to increase water channel expression. **C,** Exogenous administration of desmopressin, an ADH analogue, can replace the deficiency of posterior pituitary gland-derived ADH, and, thereby, treat neurogenic diabetes insipidus. **D,** In nephrogenic diabetes insipidus, the $V_2$ receptor gene is mutated, and the $V_2$ receptor is missing or unresponsive to stimulation by ADH. The lack of functional $V_2$ receptors prevents the cell from responding to ADH with an increase in water channel expression. There is currently no specific pharmacologic intervention for treatment of nephrogenic diabetes insipidus.

roles of oxytocin mediate muscular contraction; two well-documented effects of oxytocin are milk release during lactation and uterine contraction. In the milk letdown response, stimuli to the hypothalamus cause oxytocin release into blood from nerve terminals in the posterior pituitary gland. Oxytocin causes contraction of myoepithelial cells surrounding the mammary gland alveoli. This is an important physiologic action during breast feeding. In addition, it has long been known that administration of oxytocin causes uterine contraction. Oxytocin release is probably not the physiologic stimulus for initiation of labor during pregnancy; however, oxytocin is used pharmacologically to induce labor artificially.

## Conclusion and Future Directions

Hormones of the hypothalamus and pituitary gland can be used as pharmacologic agents to modify the respective endocrine axes of each hormone. Hypothalamic hormones can be used to determine the causes of underlying endocrine pathology diagnostically (CRH, GHRH, TRH), or to suppress an axis (GnRH, somatostatin). Hormones of the anterior pituitary gland can be given as replacement in cases of deficiency (growth hormone) or diagnostically (ACTH, TSH). The posterior pituitary gland produces two hormones, ADH and oxytocin, which can be used for treatment of neurogenic diabetes insipidus and to induce labor, respectively. Future directions in hypothalamic and pituitary gland pharmacology will include design of new drug delivery systems, such as nasal growth hormone secretagogue sprays, synthesis of orally active, nonpeptide analogues of hypothalamic hormones, development of ghrelin antagonists, and new pharmacologic interventions for nephrogenic diabetes insipidus.

## Suggested Readings

Broglio F, Gottero C, Arvat E, Ghigo E. Endocrine and nonendocrine actions of ghrelin. Horm Res 2003;59:109–117. (*Review of recent developments in ghrelin and GHS-R research*)

Lamberts S. Octreotide. N Engl J Med 1998;334:246–254. (*Clinical information regarding octreotide*)

Roith D. Insulin-like growth factors. N Engl J Med 1997;336:633–640. (*Excellent discussion of disturbances of growth hormone activity*)

Vance M, Mauras N. Growth hormone therapy in adults and children. N Engl J Med 1999;341:1206–1216. (*Clinical information on growth hormone replacement*)

## Drug Summary Table Pharmacology of the Hypothalamus and Pituitary Gland

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **GROWTH HORMONE REPLACEMENT**<br>*Mechanism—Stimulate release of or replace growth hormone* | | | | |
| **Growth hormone** | Growth hormone deficiency<br>AIDS cachexia<br>Turner's syndrome | Intracranial hypertension<br>Hyperglycemia, pancreatitis | Never use if patient has intracranial tumor | Caution in diabetes<br>Available as depot injection |
| **Sermorelin (GHRH)** | Diagnostic evaluation of ↓ plasma growth hormone | Chest tightness, transient flushing | Do not use with another drug that affects pituitary gland | Also approved as growth hormone therapy in children with tertiary (hypothalamic) deficiency |
| **Hexarelin**<br>**Ghrelin** | Investigational | Undetermined | Undetermined | Experimental agents that bind to GH-secretagogue receptor<br>Hexarelin formulations available intranasally |
| **AGENTS THAT DECREASE GROWTH HORMONE LEVELS**<br>*Mechanism—Inhibit GHRH release* | | | | |
| **Octreotide**<br>**Somatostatin** | Acromegaly<br>VIPomas and carcinoid tumors | Dizziness, flushing | Decrease cyclosporine levels | Also used to control GI bleeding and to reduce secretory diarrhea |
| **AGENTS THAT DECREASE PROLACTIN LEVELS**<br>*Mechanism—Inhibition of pituitary prolactin release* | | | | |
| **Bromocriptine** | Prolactinoma | Nausea, postural hypotension | Hypersensitivity to ergots | Ergot alkaloid. Dose 2×/day |
| **Pergolide** | Prolactinoma | Nausea, postural hypotension | Hypersensitivity to ergots | Ergot alkaloid. Dose 1×/day. Also used in Alzheimer's disease |
| **Quinoglide** | Prolactinoma | Nausea, postural hypotension | Hypersensitivity to ergots | Nonergot. Not available in USA |
| **Cabergoline** | Prolactinoma | Nausea, postural hypotension | Hypersensitivity to ergots | Dose 1×/week. Binds to D2 receptor.<br>Nonergot; less nausea than bromocriptine or pergolide |

*(Continued)*

**Drug Summary Table** **Pharmacology of the Hypothalamus and Pituitary Gland** *(Continued)*

| Drug | Clinical Use | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **AGENTS THAT TEST THYROID FUNCTION**<br>*Mechanism—TRH stimulates TSH release from pituitary; TSH stimulates thyroid gland* | | | | |
| **Protirelin (TRH)**<br>**Thyrotropin (TSH)** | Dx thyroid function | Anxiety, diaphoresis | Known hypersensitivity | |
| **AGENTS THAT TEST ADRENAL FUNCTION**<br>*Mechanism—Stimulates adrenal cortisol and androgen production* | | | | |
| **Corticotropin** | Dx adrenal insufficiency | | | Also used in multiple sclerosis to decrease autoimmune inflammatory response |
| **AGENTS THAT ALTER GONADOTROPIN EXPRESSION**<br>*Mechanism—Continuous: inhibit LH and FSH release; Pulsatile: stimulate LH and FSH release* | | | | |
| **Gonadorelin** | Diagnostic<br>Stimulate ovulation | | | Pulsatile form for stimulation of ovulation |
| **Goserelin**<br>**Histrelin**<br>**Leuprolide**<br>**Nafarelin** | All 4 agents can be used in:<br>Prostate cancer<br>Endometriosis<br>Precocious puberty<br>Acute intermittent porphyria | All 4 agents can cause:<br>Hot flashes (men and women)<br>Osteoporosis<br>Sexual dysfunction | All 4 agents can initially increase testosterone and estrogen levels | Depot formulations that result in gonadotropin suppression and consequent ↓ gonadal steroids |
| **Follitropin (rFSH)**<br>**Urofollitropin (FSH)** | Ovulation induction | Ovarian hyperstimulation syndrome | Any endocrine disorder other than anovulation | May result in multiple fetuses |

# 25

# Pharmacology of the Thyroid Gland

Ehrin J. Armstrong, Armen H. Tashjian, Jr., and William W. Chin

- Introduction
- Case
- Thyroid Gland Physiology
  - Synthesis and Secretion of Thyroid Hormones
  - Metabolism of Thyroid Hormone
  - Effects of Thyroid Hormones on Target Tissues
  - Hypothalamic-Pituitary–Thyroid Axis
- Pathophysiology
- Pharmacologic Classes and Agents
  - Treatment of Hypothyroidism
  - Treatment of Hyperthyroidism
    - *Inhibitors of Iodide Uptake*
    - *Inhibitors of Organification and Hormone Release*
    - *Inhibitors of Peripheral Thyroid Hormone Metabolism*
  - Other Drugs Affecting Thyroid Hormone Homeostasis
    - *Lithium*
    - *Amiodarone*
    - *Corticosteroids*
- Conclusion and Future Directions
- Suggested Readings

## INTRODUCTION

The thyroid gland has diverse and important effects on many aspects of metabolic homeostasis. **Follicular thyroid cells** constitute the majority of thyroid tissue; these cells produce and secrete the classical thyroid hormones discussed in this chapter: thyroxine (T4), triiodothyronine (T3), and reverse triiodothyronine (rT3). Thyroid hormones regulate energy metabolism and growth, from oxygen consumption to cardiac contractility. **Parafollicular C cells** of the thyroid gland secrete **calcitonin,** a regulator of bone mineral homeostasis. Calcitonin is discussed in Chapter 29, Pharmacology of Bone Mineral Homeostasis.

The major diseases of the thyroid gland involve disruption of the normal hypothalamic-pituitary–thyroid axis (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). Replacement of deficient thyroid hormone is an effective and established therapy for hypothyroidism. Treatment of hyperthyroidism is more complex, with options ranging from antithyroid drugs to surgical excision of abnormal tissue. Understanding the pathways and mechanisms of feedback regulation of thyroid hormone synthesis and thyroid hormone actions serves to explain the rationale for effective drug treatment of thyroid diseases.

## ■ Case

Over the course of a few months, Diana Lau, 45 years old, notices a number of disconcerting changes in the way she feels and her general appearance. Ms. Lau feels nervous all the time; small events make her jumpy. She also keeps the temperature unusually cold in her house, to the point where her husband and children begin to complain. Because of these symptoms and the occasional feeling that her heart "skips a beat," Ms. Lau goes to see her doctor. After some questioning, he palpates her neck and notes that her thyroid gland is diffusely enlarged. He also notes that Ms. Lau's eyes are more prominent than normal. Tests for thyroid hormone levels reveal high serum-free triiodothyronine (T3) and low thyrotropin (TSH). In addition, a test for TSH receptor antibody is positive. Ms. Lau is treated with radioactive iodide. Although initially comforted by the fact that her doctor has some answers, she soon becomes discouraged because she does not notice any changes in her symptoms for a couple of weeks. After a month, however, her symptoms begin to subside. Four years later, however, she begins to develop symptoms that are the opposite of her original problems: she feels tired all the time, and she gains 30 pounds over the course of

6 months. Her doctor confirms that Ms. Lau has developed hypothyroidism. She now takes thyroxine (T4) once per day.

## QUESTIONS

1. Why was Ms. Lau's serum thyrotropin level low, but her triiodothyronine concentration high?
2. What features of the thyroid gland make radioactive iodide a generally safe and specific therapy for hyperthyroidism?
3. Why did Ms. Lau develop hypothyroidism after treatment with radioactive iodide?

## THYROID GLAND PHYSIOLOGY

### SYNTHESIS AND SECRETION OF THYROID HORMONES

The thyroid is an endocrine gland located in the neck inferior to the larynx and spanning the ventral surface of the trachea. The main function of the thyroid gland is to produce the thyroid hormones, T3 and T4. Structurally, the thyroid hormones are built on a backbone of two tyrosine molecules that are iodinated and connected by an ether linkage (Fig. 25-1). An important structural feature of thyroid hormones is the placement of iodines on this backbone. The position and relative orientation of iodines attached to the tyrosine residues determines the specific form of thyroid hormone. **3,5,3′,5′-Tetraiodothyronine (thyroxine, T4)** has four iodines attached to the tyrosine backbones and is the main form of thyroid hormone secreted by the thyroid gland. **3,5,3′-Triiodothyronine (T3)** has three iodines. Most T3 is produced by peripheral 5′ deiodination of T4 (see below). Biologically inactive forms of thyroid hormone include 3,3′,5′-triiodothyronine, also referred to as **reverse triiodothyronine (rT3),** because the single iodine is on the opposite tyrosine in the backbone relative to T3. Circulating thyroid hormone in a normal individual consists of about 90% T4, 9% T3, and 1% rT3.

Iodide is a trace element that is a crucial component of thyroid hormone structure. Thyroid follicular cells, which synthesize and secrete thyroid hormones, selectively concentrate iodide via an $Na^+/I^-$ symporter located on the basolateral membrane of the follicular cell (Fig. 25-2). This active transport mechanism has the ability to concentrate iodide to intracellular concentrations up to 500 times that of plasma; most individuals have thyroid gland to plasma iodide ratios of approximately 30.

Once inside thyroid gland follicular cells, iodide is secreted through the follicular cell apical membrane and concurrently oxidized by the enzyme **thyroid peroxidase** (Fig. 25-2). This oxidation reaction creates a more reactive iodide intermediate that couples to specific tyrosine residues on **thyroglobulin.** Thyroglobulin is a protein synthesized by thyroid follicular cells and secreted at the apical surface into the colloid space. Thyroid peroxidase is also concentrated at the apical surface, and it is thought that generation of oxidized iodide at this surface allows the iodide to react with tyrosine residues in newly secreted thyroglobulin molecules. This process of iodination of thyroglobulin is known as **organification.** Organification results in thyroglobulin molecules containing **monoiodotyrosine** (MIT) and **diiodotyrosine** (DIT) residues, depending on whether each tyrosine has one or two iodines covalently attached, respectively.

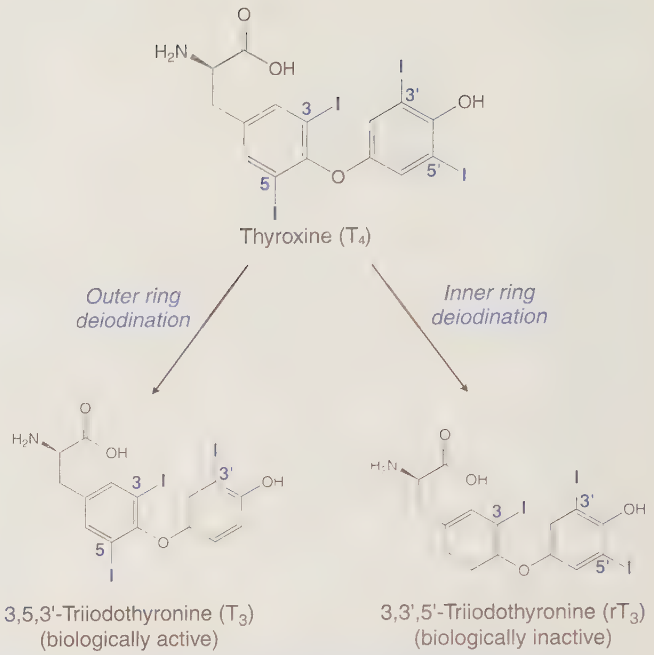

O
H₂N
OH
I
3
3'
OH
I
5
5'
I
O
I
Thyroxine (T₄)
Outer ring deiodination
Inner ring deiodination
3,5,3'-Triiodothyronine (T₃) (biologically active)
3,3',5'-Triiodothyronine (rT₃) (biologically inactive)

**Figure 25-1. Structure and Peripheral Metabolism of Thyroid Hormones.** Thyroid hormones are synthesized from two derivatized tyrosine molecules that are attached by an ether linkage. The outer ring is hydroxylated; whereas, the inner ring is linked covalently to thyroglobulin during thyroid hormone synthesis. Iodine is attached to three or four positions of the tyrosine backbone to create several different substitution patterns. Thyroxine (T4) has four iodines attached, two on each ring. Thyroxine is the predominant thyroid hormone produced by the thyroid gland. Triiodothyronine (T3) has two iodines attached to the inner ring, but only one iodine attached to the outer ring. In contrast, reverse triiodothyronine (rT3) has two iodines on the outer ring, but only one iodine on the inner ring. During peripheral metabolism, thyroxine is deiodinated by 5′-deiodinases present in target tissues and in liver. The pattern of deiodination produces either T3 or rT3. If the iodine is removed from the outer ring, the biologically active T3 is produced. If the iodine is removed from the inner ring, the biologically inactive rT3 is produced.

After generation of MITs and DITs within thyroglobulin, thyroid peroxidase also catalyzes **coupling** between these residues. An MIT joined to DIT generates T3, while joining two DITs creates T4. Note that nascent T3 and T4 are still covalently part of the thyroglobulin protein at this point. These thyroglobulin molecules are then stored in the lumen of the follicle as **colloid.**

When thyroid-stimulating hormone (discussed below) signals the thyroid gland to secrete thyroid hormone, the follicular cells endocytose colloid. The ingested thyroglobulin enters lysosomes, where proteases digest the thyroglobulin. Proteolytic digestion releases free T3, T4, MIT, and DIT. T3 and T4 are transported across the follicular cell basolateral membrane and into the bloodstream. Free MIT and DIT are rapidly deiodinated within the cell so that the iodide can be recycled for new thyroid hormone synthesis.

Most endocrine organs concurrently synthesize and release new hormone when activated, rather than storing large quantities of precursor hormone. The *thyroid gland is unusual among*

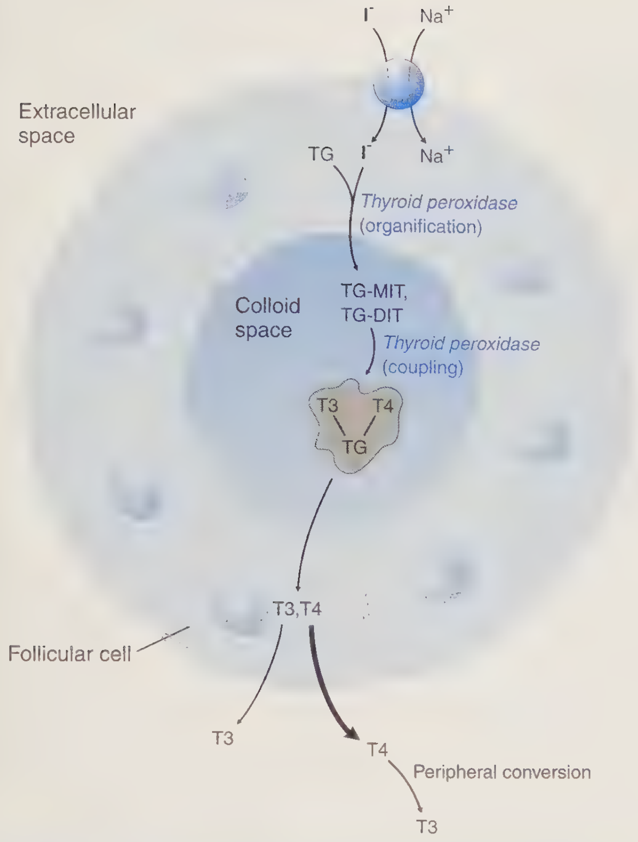


**Figure 25-2. Thyroid Hormone Synthesis, Storage, and Release.** Follicular cells of the thyroid gland concentrate iodide ($I^-$) from plasma via a basolateral membrane $Na^+/I^-$ symporter. In a reaction (called "organification") catalyzed by thyroid peroxidase, intracellular iodide reacts covalently with tyrosine residues on thyroglobulin (TG) molecules at the apical membrane. Addition of one $I^-$ to tyrosine results in the formation of monoiodinated tyrosine (MIT); addition of two $I^-$ to tyrosine results in the formation of diiodinated tyrosine (DIT). MIT and DIT associate covalently on thyroglobulin via a mechanism known as "coupling," which is also catalyzed by thyroid peroxidase. The derivatized thyroglobulin is stored as colloid within follicles in the thyroid gland. Upon stimulation by TSH, thyroid follicular cells endocytose colloid into lysosomal compartments, where the thyroglobulin is degraded to yield free T4, free T3, and uncoupled MIT and DIT. T3 and T4 are secreted into the plasma, and MIT and DIT are deiodinated intracellularly to yield free iodide for use in new thyroid hormone synthesis (not shown). The thyroid gland secretes more T4 than T3, although T4 is converted to T3 in peripheral tissues.

*endocrine glands in that it stores large quantities of thyroid prohormone in the form of thyroglobulin.* It is not understood why the thyroid gland maintains this elaborate pathway for hormone synthesis and release, but doing so may make it possible to maintain plasma thyroid hormone at a constant level despite fluctuations in the availability of dietary iodide.

## METABOLISM OF THYROID HORMONE

Thyroid hormone circulates bound to plasma proteins, most notably **thyroid binding globulin (TBG)** and transthyretin. Although T4 is the predominant thyroid hormone found in the blood, T3 has four times the physiologic activity of T4 on target tissues. Some serum T4 is inactivated by deamination, decarboxylation, or conjugation and excretion by the liver. Most T4, however, is deiodinated to the more active T3 form in several locations in the body. This deiodination reaction is catalyzed by the enzyme **iodothyronine 5′-deiodinase** (Fig. 25-1).

There are three subtypes of 5′-deiodinase. **Type I deiodinase,** expressed in the liver and kidneys, is important for converting T4 to the majority of serum T3. **Type II deiodinase** is expressed primarily in the pituitary, brain, and brown fat. This enzyme is located intracellularly and can convert T4 into T3 locally. Finally, **type III deiodinase** is responsible largely for T4 and T3 degradation.

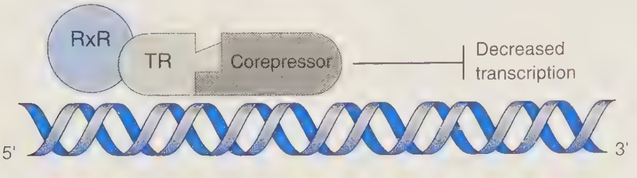
The presence of T4 in the blood provides a buffer, or reservoir, for thyroid hormone effects. Most T4 to T3 conversion occurs in the liver, and many pharmacologic agents that increase hepatic microsomal enzyme activity will increase T4 to T3 conversion. In addition, T4 has a half-life in the plasma of approximately 6 days, whereas, plasma T3 has a half-life of only 1 day. *Because T4 has a long plasma half-life, changes in thyroid hormone-regulated functions caused by pharmacologic intervention are generally not observed for a period of 1 to 2 weeks,* as seen with Ms. Lau in the introductory case.

## EFFECTS OF THYROID HORMONES ON TARGET TISSUES

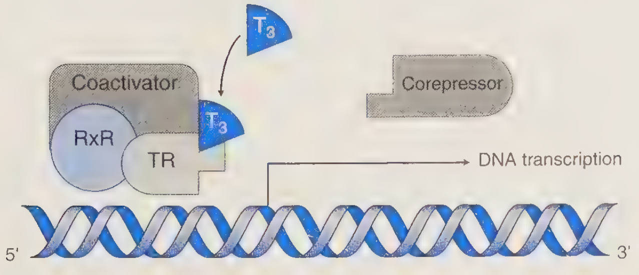
Thyroid hormones have effects on virtually every cell of the body. Free hormone diffuses into most cell types, where it then binds to intracellular thyroid hormone receptors (TRs). TRs are proteins with thyroid hormone-binding, DNA-binding, and dimerization domains. There are two classes of thyroid receptor, termed **TRα** and **TRβ.** In addition, both TRα and TRβ can be expressed as multiple isoforms. TR monomers can interact in a dimerization reaction to form homodimers, or with another transcription factor, **retinoic acid X receptor** (RXR), to form heterodimers. These TR heterodimers bind to gene promoter regions and are activated by binding of thyroid hormones. Together, the multiple different possible combinations of TRs and the variability in their tissue distributions create tissue specificity for thyroid hormone effects.

In the absence of hormone, thyroid hormone receptor dimers associate with co-repressor molecules and constitutively bind to (and, thereby, inactivate) thyroid hormone-stimulated genes. Binding of thyroid hormone to TR dimers promotes dissociation of the co-repressors and recruitment of co-activators to the DNA. Thus, thyroid hormone binding to TR dimers serves as a molecular switch from inhibition to activation of gene transcription (Fig. 25-3). Thyroid hormone also acts to down-regulate gene expression by a TR-dependent mechanism, the exact nature of which is not fully understood. For example, thyroid hormone is able to down-regulate TSH gene expression, causing negative feedback of thyroid hormone upon the hypothalamic-pituitary–thyroid axis (see Chapter 24).

Thyroid hormone is important in infancy for growth and development of the nervous system. Congenital deficiency of thyroid hormone results in **cretinism,** a severe but preventable form of mental retardation. In the adult, thyroid hormone regulates general body metabolism and energy expenditure. Some enzymes regulated by thyroid hormone include the $Na^+/K^+$ ATPase and many of the enzymes of intermediary metabolism, both anabolic and catabolic. At high levels of thyroid hormone, this effect can result in futile



No thyroid hormone



With thyroid hormone

**Figure 25-3. Thyroid Hormone Receptor Actions.** In the absence of thyroid hormone, the thyroid hormone receptor (TR):retinoic acid receptor (RXR) heterodimer associates with a co-repressor complex, which binds to promoter regions of DNA and inhibits gene expression. In the presence of thyroid hormone (T3), the co-repressor complex dissociates from the TR:RXR heterodimer, coactivators are recruited, and gene transcription occurs. This example demonstrates the action of T3 on a TR:RXR heterodimer, but similar mechanisms are probable for TR:TR homodimers. A useful strategy in the future may involve pharmacologic targeting of tissue-specific co-repressors or co-activators.

cycling with a consequent increase in body temperature—this is why Ms. Lau was always turning down the heat in her home. Many of the effects of thyroid hormone resemble the effects of sympathetic stimulation, including increased cardiac contractility and heart rate, excitability, nervousness, and diaphoresis (sweating). These symptoms were also seen in Ms. Lau—she was nervous all the time and was startled by slight provocations. Conversely, low levels of thyroid hormone activity result in lethargy and myxedema (a hypometabolic state with lethargy, dry skin, coarse voice, and cold intolerance).

## HYPOTHALAMIC-PITUITARY–THYROID AXIS

Thyroid hormone secretion follows a negative regulatory feedback scheme similar to that of other hypothalamic-pituitary–target organ axes (Fig. 25-4). **Thyrotropin-releasing hormone (TRH)** is a tripeptide secreted by the hypothalamus that travels via the hypothalamic-hypophyseal portal system to the anterior pituitary gland (see Chapter 24). TRH binds to a G protein-coupled receptor present on the membrane of anterior pituitary gland thyrotrophs, or TSH-producing cells. This stimulates a signal transduction cascade that ultimately promotes the synthesis and release of **thyroid-stimulating hormone (TSH).** TSH is the most important direct regulator of thyroid gland function. TSH stimulates every known aspect of thyroid hormone production, including iodide uptake, organification, coupling, and secretion of thyroid hormone. In addition, TSH promotes increased vascularization and growth of the thyroid gland. In pathologic conditions where TSH or a TSh mimic (see below) is secreted at high levels, the thyroid gland can enlarge to several times its normal size, resulting in the characteristic diffusely hypertrophied thyroid gland referred to as a **goiter,** which Ms. Lau's doctor noted when he palpated her neck.

Negative feedback of the hypothalamic-pituitary–thyroid axis occurs through regulatory action of thyroid hormone on both the hypothalamus and pituitary gland. Secreted thyroid hormone diffuses into the thyrotrophs of the anterior pituitary gland, where it binds to and activates nuclear thyroid hormone receptors. These receptors then inhibit TSH gene transcription and, hence, synthesis of TSH. Thyroid hormone also has important regulatory effects on the hypothalamus itself; binding of thyroid hormone to receptors in hypothalamic cells inhibits transcription of the gene that codes for the TRH precursor protein.

## PATHOPHYSIOLOGY

The pathophysiology of thyroid diseases can be understood as a disturbance of the physiologic hypothalamic-pituitary–thyroid axis. For example, increases in thyroid hormones normally suppress TSH synthesis and release and, thereby suppress the endocrine axis. Pathologic thyroid hormone insufficiency reduces the negative feedback of thyroid hormones on TSH release. Consequently, TSH levels are elevated without a concurrent rise in thyroid hormone release.

Most common thyroid diseases are best categorized as conditions that result in increased (hyperthyroid) or decreased (hypothyroid) thyroid hormone secretion. Two common thyroid diseases are **Graves' disease** and **Hashimoto's thyroiditis** (Fig. 25-4). Each is believed to be autoimmune in origin, but Graves' disease causes hyperthyroidism, whereas, Hashimoto's thyroiditis results in hypothyroidism.

Graves' disease demonstrates the importance of plasma thyroid hormone in regulating homeostasis of the hypothalamic-pituitary–thyroid axis. In this syndrome, an IgG autoantibody specific for the TSH receptor, known as **thyroid-stimulating immunoglobulin (TsIg),** is produced. This antibody activates the TSH receptor and stimulates thyroid follicular cells to synthesize and release thyroid hormone. *Unlike TSH, however, TsIg is not subject to negative feedback; it continues to stimulate thyroid function even when plasma thyroid hormone levels rise to pathologic levels.* Because the autoantibody in Graves' disease acts independently of the hypothalamic-pituitary–thyroid axis, thyroid hormone homeostasis is disrupted; clinical findings include symptoms of hyperthyroidism, with laboratory findings of high plasma thyroid hormone concentration but low or undetectable levels of TSH. In the introductory case, Ms. Lau's TSH levels were low because the peripheral excess of thyroid hormone suppressed anterior pituitary gland release of TSH.

Hashimoto's thyroiditis, in contrast, results in selective destruction of the thyroid gland. Antibodies specific for many thyroid gland proteins, including thyroglobulin and thyroid peroxidase, can be found in the plasma of patients with Hashimoto's thyroiditis. As with Graves' disease, the underlying etiology of this disease is thought to be autoimmune in nature. The clinical course of Hashimoto's thyroiditis involves a gradual inflammatory destruction of the thyroid

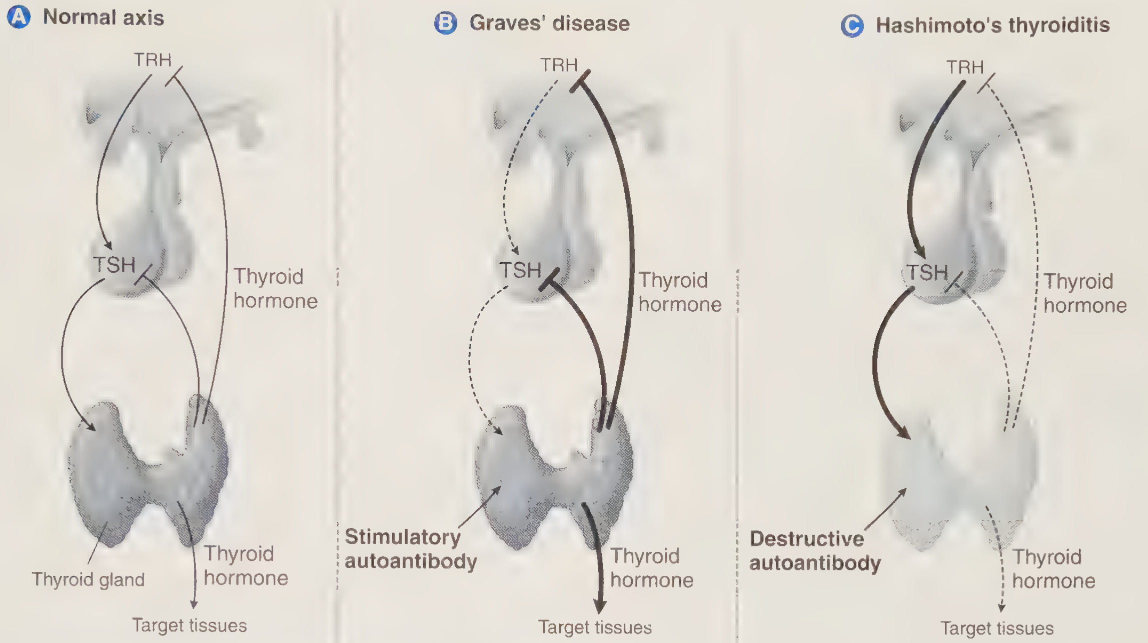


Figure 25-4. The Hypothalamic-Pituitary–Thyroid Axis in Health and Disease. **A,** In the normal axis, thyrotropin-releasing hormone (TRH) stimulates thyrotropes of the anterior pituitary gland to release thyroid-stimulating hormone (TSH). TSH stimulates synthesis and release of thyroid hormone by the thyroid gland. Thyroid hormone, in addition to its effects on target tissues, inhibits further release of TRH and TSH by the hypothalamus and anterior pituitary gland, respectively. **B,** In Graves' disease, a stimulatory autoantibody autonomously activates the TSH receptor in the thyroid gland, resulting in sustained stimulation of the thyroid gland, increased plasma thyroid hormone (thick lines), and suppression of TRH and TSH release (dashed lines). **C,** In Hashimoto's thyroiditis, a destructive autoantibody attacks the thyroid gland, causing thyroid insufficiency and decreased synthesis and secretion of thyroid hormone (dashed lines). Consequently, feedback inhibition on the hypothalamus and anterior pituitary gland does not occur, and plasma TSH levels rise (thick lines).

gland with resultant hypothyroidism. Early in the course of the disease, destruction of thyroid follicular cells can release excessive quantities of stored colloid, resulting in transiently increased levels of thyroid hormone. Eventually, the gland is almost completely destroyed, and clinical symptoms of hypothyroidism, such as lethargy and decreased metabolic rate, occur. Therapy for Hashimoto's thyroiditis involves pharmacologic replacement with oral synthetic thyroid hormone.

Other causes of hypothyroidism and hyperthyroidism exist, including developmental anomalies, subacute (DeQuervain) thyroiditis, thyroid adenomas, and carcinomas. Details of the underlying pathophysiologies differ, but pharmacologic intervention in each case rests on determining whether the patient is hypothyroid, euthyroid, or hyperthyroid.

## PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic treatment of thyroid gland pathophysiology involves either replacement of deficient thyroid hormone or antagonism of excessive thyroid hormone. Replacement is self-evident, while the antagonists work at multiple steps in thyroid hormone synthesis and action (Fig. 25-5). In addition, a number of pharmacologic agents used for nonthyroid disease indications have important effects on peripheral thyroid hormone metabolism. The mechanisms of their action are discussed at the end of this section.

### TREATMENT OF HYPOTHYROIDISM

Use of thyroid hormone is a well-established and safe therapy for long-term treatment of hypothyroidism. Therapy aims to replace missing endogenous thyroid hormone with regularly administered exogenous thyroid hormone. The thyroid hormone administered is structurally identical to the endogenous thyroid hormone (generally T4) and is produced by chemical synthesis.

Early trials with replacement hormone debated whether it would be more efficacious to provide replacement of T3 or T4. T3 is the metabolically more active form of thyroid hormone, so one might anticipate that replacement of deficient thyroid hormone with T3 would more effectively normalize thyroid homeostasis. However, a number of findings argue against this. First, most thyroid hormone in the blood is T4, although T4 has lower activity and is eventually metabolized to T3. Having a large reservoir of thyroid "prodrug" (T4) in the plasma may be important, perhaps as an effective buffer to normalize metabolic rates over a wide range of conditions. Second, the half-life of T4 is 6 days, as compared to the 1-day half-life of T3. The extended half-life of T4 allows a patient to take just one thyroid hormone replacement pill per day. For these reasons, **levothyroxine,** the L-isomer of T4, is the treatment of choice for hypothyroidism. The efficacy of thyroid hormone replacement is monitored by assay of plasma TSH levels and thyroid hormone concentrations. TSH is an accurate marker of thyroid hormone activity because anterior pituitary gland release of TSH is exquisitely

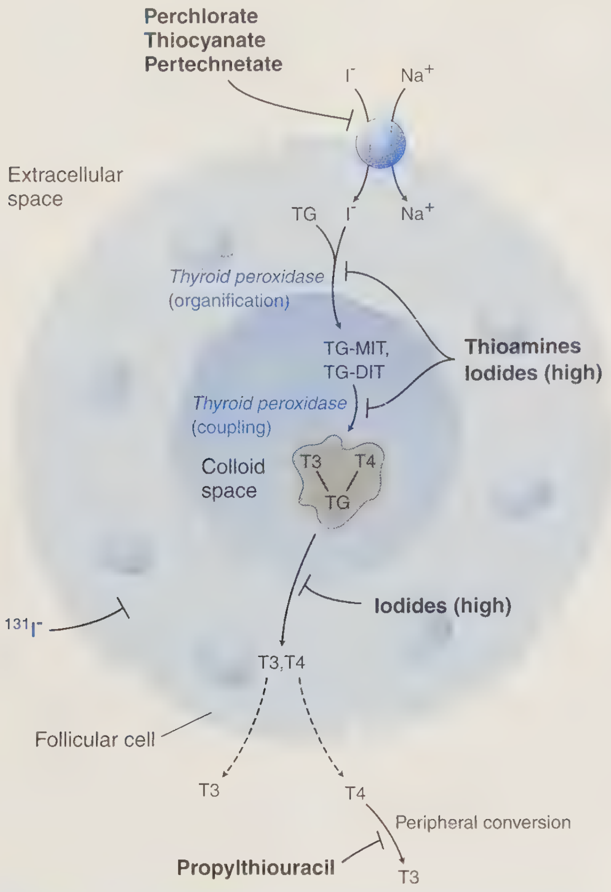


**Figure 25-5. Pharmacologic Interventions Affecting Thyroid Hormone Synthesis.** Anions with molecular radii approximately equal to that of the iodide anion ($I^-$), such as perchlorate, thiocyanate, and pertechnetate, compete with iodide for uptake by the $Na^+/I^-$ symporter. Radioactive $^{131}I^-$, when concentrated within thyroid cells, causes selective destruction of the thyroid gland. High levels of iodide transiently depress thyroid function by inhibiting organification, coupling, and proteolysis of thyroglobulin. Thioamines, such as propylthiouracil and methimazole, inhibit organification and coupling; propylthiouracil also inhibits peripheral conversion of T4 to T3. TG-MIT, thyroglobulin-monoiodotyrosine; TG-DIT, thyroglobulin-diiodotyrosine.

sensitive to feedback control by thyroid hormone levels in the blood.

## TREATMENT OF HYPERTHYROIDISM

There are pharmacologic agents that target each step of thyroid hormone synthesis, from initial uptake of iodide, to organification, coupling, and peripheral conversion of T4 to T3. Clinically, both radioactive iodide and thioamines are available for treatment of hyperthyroidism. β-adrenergic antagonists are also sometimes used to ameliorate some of the symptoms of hyperthyroidism.

### Inhibitors of Iodide Uptake

Iodide, as mentioned above, is brought into the cell via a $Na^+/I^-$ symporter. Certain anions with the approximate atomic radius of iodide, such as **perchlorate, thiocyanate,** and **pertechnetate,** compete with iodide for uptake into the thyroid gland follicular cell (Fig. 25-5). This results in decreased iodide available for thyroid hormone synthesis. The effects of anion uptake inhibitors are usually not apparent for over a week, because of the large store of thyroid hormone in the colloid.

Anion uptake inhibitors can be used in the treatment of hyperthyroidism; these agents reduce the intrathyroidal supply of iodide available for thyroid hormone synthesis. However, their use is uncommon because of the potential for causing aplastic anemia, and the thioamines (see below) are generally more effective. Because many of these agents are used as radiopaque contrast materials, it is important to keep this physiologic antagonism in mind whenever a patient has symptoms of hypothyroidism after extensive radiographic studies employing contrast material.

### Inhibitors of Organification and Hormone Release

#### *Iodides*

Two distinct types of iodide are used in clinical practice. Both take advantage of the thyroid gland's selective uptake and concentration of iodide to levels much higher than that in the blood.

The first agent, $^{131}I^-$, is a radioactive iodide isotope that strongly emits β-rays toxic to cells. The $Na^+/I^-$ channel expressed on thyroid follicular cell membranes cannot distinguish $^{131}I^-$ from normal stable iodide ($^{127}I^-$). Therefore, $^{131}I^-$ becomes sequestered within the thyroid gland. This makes radioactive $^{131}I^-$ a specific and effective therapy for hyperthyroidism. The concentrated intracellular radioactive iodide continues to emit β-rays, resulting in selective destruction of the thyroid gland. Radioactive iodide is used to treat thyrotoxicosis; this agent serves as an alternative to surgery in the treatment of hyperthyroidism. There is a concern that patients may eventually develop hypothyroidism after treatment with radioactive iodide because it is difficult to ascertain for a given patient the extent to which radioactive $^{131}I^-$ will kill his or her thyroid follicular cells. *The goal is to administer enough $^{131}I^-$ to result in a euthyroid state, without precipitating hypothyroidism.* This desired result is not always obtained; for example, Ms. Lau developed hypothyroidism after treatment with $^{131}I^-$. Regardless, development of hypothyroidism is easier to manage clinically than hyperthyroidism. Although unlikely, it is also important to determine whether radioactive iodide treatment has other long-term side effects, such as thyroid cancer.

The second clinically important pharmacologic agent is, interestingly, stable inorganic iodide. High levels of iodide inhibit thyroid gland hormone synthesis and release, a phenomenon known as the **Wolff–Chaikoff effect.** The negative feedback effect of high intrathyroidal iodide concentrations is reversible and transient; thyroid hormone synthesis and release returns to normal a few days after the plasma iodide concentration is increased. Therefore, inorganic iodide is not a useful long-term therapy for hyperthyroidism. This phenomenon does, however, have other important uses. For example, high iodide dosing reduces the size and vascularity of the thyroid gland. Because of this, iodide is often administered before thyroid gland surgery, resulting in technically easier excision of the gland.

Iodide can also have important preventative effects. When the nuclear accident at Chernobyl occurred, there was concern that radioactive iodide released into the air over Poland could cause population-wide thyroid gland destruction. As a preventative measure, millions of Polish children were given large doses of iodide for a number of days to suppress thyroid gland function temporarily and, thereby, avoid uptake of radioactive iodide.

### *Thioamines*

Thioamines are useful inhibitors of thyroid hormone production. Thioamines compete with thyroglobulin for oxidized iodide in a process that is catalyzed by the enzyme thyroid peroxidase (Fig. 25-5). This step is important for organification and coupling of thyroid hormone precursors. *By competing for oxidized iodide, thioamine treatment causes a selective decrease in thyroid hormone production.* Iodinated thioamines may also be able to bind to thyroglobulin, further antagonizing any coupling reactions. Recall that thyroid follicular cells store a large quantity of thyroid hormone–thyroglobulin protein as colloid. This colloid can provide enough thyroid hormone for over a week in the absence of any new synthesis. Because thioamines affect the synthesis rather than secretion of thyroid hormone, the effects of these drugs are not seen until several weeks after thyroid gland stores are depleted.

Thioamines are commonly referred to as **goitrogens** because inhibition of thyroid hormone production results in upregulation of TSH production by the anterior pituitary gland, and, hence, in thyroid gland stimulation, in an attempt to reestablish homeostasis. The increased plasma TSH cannot raise thyroid hormone levels because of the action of the thioamine, however. In response to the elevated TSH, the thyroid gland hypertrophies in an attempt to increase synthesis of new thyroid hormone. This results in the eventual formation of a goiter.

**Propylthiouracil** is considered the prototype thioamine; **methimazole** is another frequently used drug in this class. Propylthiouracil inhibits thyroid peroxidase as well as peripheral T4 to T3 conversion; whereas, methimazole has only been shown to inhibit thyroid peroxidase.

Both propylthiouracil and methimazole cause rashes and arthalgias with some frequency, resulting in a lupus-like syndrome. Propylthiouracil can also deplete levels of prothrombin, leading to hypoprothrombinemia and an increased bleeding tendency. Both propylthiouracil and methimazole cross the placenta, and methimazole has been associated with rare case reports of birth defects. Methimazole is also more potent than propylthiouracil, making it more likely to cause fetal hypothyroidism. For both these reasons, propylthiouracil is the preferred agent for treatment of hyperthyroidism in pregnancy.

## Inhibitors of Peripheral Thyroid Hormone Metabolism

Although the majority of thyroid hormone is synthesized in the thyroid gland as T4, the majority of thyroid hormone acts at peripheral sites as T3. Conversion of T4 to T3 is dependent on a peripheral 5′-deiodinase, and inhibitors of this enzyme are effective adjuncts for treatment of the symptoms of hyperthyroidism. As mentioned above, propylthiouracil inhibits both organification and peripheral conversion of T4 to T3. Two other agents, β-adrenergic blockers and ipodate, are discussed below.

### *β-Adrenergic Blockers*

β-Adrenergic antagonists are useful therapies for treating symptoms of hyperthyroidism because many of the effects of high plasma thyroid hormone levels resemble nonspecific β-adrenergic stimulation (e.g., sweating, tremor, tachycardia). In addition, it has been demonstrated that β-blockers can reduce T4/T3 conversion of thyroid hormone, but this effect is not thought to be clinically relevant. **Esmolol** is a preferred β-adrenergic antagonist for treatment of thyroid storm because of its rapid onset of action and short elimination half-life (9 minutes).

### *Ipodate*

Ipodate is a radiocontrast agent used for visualization of the biliary ducts in endoscopic retrograde cholangiopancreatography (ERCP) procedures. In addition to its usefulness as a radiocontrast agent, ipodate significantly inhibits conversion of T4 to T3 by inhibiting the enzyme 5′-deiodinase. Trials have been performed demonstrating that use of both ipodate and methimazole together is more effective in controlling hyperthyroidism than methimazole alone. Care must be taken when administering ipodate to a patient with hyperthyroid symptoms because ipodate contains large quantities of iodine. If the patient has a toxic adenoma or goiter, addition of this excess iodine to the thyroid can result in worsening of hyperthyroid symptoms. In euthyroid individuals, the large iodide load can cause suppression of thyroid gland activity by the Wolff-Chaikoff effect.

# OTHER DRUGS AFFECTING THYROID HORMONE HOMEOSTASIS

## Lithium

Lithium, a drug commonly used in the treatment of bipolar disorder (see Chapter 12, Pharmacology of Affective Disorders), can cause hypothyroidism. Lithium is actively concentrated in the thyroid gland, and high levels of lithium have been shown to inhibit thyroid hormone release from thyroid follicular cells. There is some evidence that lithium may inhibit thyroid hormone synthesis as well. The mechanism(s) responsible for these actions is unknown.

## Amiodarone

Amiodarone is an antiarrhythmic drug (see Chapter 17, Pharmacology of Cardiac Rhythm) that has numerous effects on thyroid hormone function. Amiodarone structurally resembles thyroid hormone and, as a result, contains a large concentration of iodine (each 200 mg tablet of amiodarone contains 75 mg of iodine). Metabolism of amiodarone releases this iodide, resulting in increased plasma concentrations of iodide. The increased plasma iodide is concentrated in the thyroid gland; this can result in hypothyroidism by the Wolff–Chaikoff effect.

Amiodarone can also cause hyperthyroidism. The mechanism is unknown, but amiodarone seems to increase the incidence of autoimmune thyroiditis, resulting in transient release of excess thyroid hormone from the colloid. Because of its close structural similarity to thyroid hormone, it may also act as a homologue of thyroid hormone.

In addition, amiodarone competitively inhibits type I 5′-deiodinase. This results in decreased T4/T3 conversion and increased plasma concentrations of rT3.

### Corticosteroids

Corticosteroids, such as cortisol and glucocorticoid analogues, inhibit the 5′-deiodinase enzyme that converts T4 to the metabolically more active T3. Because T4 has less physiologic activity than T3, treatment with corticosteroids reduces net thyroid hormone activity. In addition, the decreased serum T3 results in increased release of TSH. The increased release of TSH stimulates greater T4 synthesis, until the amount of T4 produced creates enough T3 to inhibit the hypothalamus. Thus, when faced with decreased peripheral T4/T3 conversion, the thyroid produces a higher level of T4 until serum T4 and T3 levels reach a new steady state.

## *Conclusion and Future Directions*

Thyroid hormone synthesis consists of a complex set of synthesis and degradation steps. This creates numerous points for pharmacologic intervention, from iodide uptake to peripheral conversion of T4 to T3. Thyroid hormone replacement is a safe and effective long-term therapy for thyroid hormone deficiencies. Numerous effective therapies exist for management of thyrotoxicosis. Radioactive iodide and thioamines are commonly used for this purpose, leading to selective destruction of the thyroid gland and antagonism of organification/coupling, respectively. Future potential therapies for diseases of the thyroid gland may focus on treating the etiology of autoimmune thyroid diseases, such as Graves' disease and Hashimoto's thyroiditis, and better defining the molecular targets of thyroid hormone action.

## *Suggested Readings*

Braverman L, Utiger R, eds. Werner and Ingbar's The Thyroid. 8th Ed. Baltimore: Lippincott Williams & Wilkins, 2000. (*Clinical information on the treatment of thyroid diseases*)

Weetman A. Graves' disease. N Engl J Med 2000;343:1236–1248. (*A very readable review of the diagnosis and treatment of Graves' disease*)

Wolf G. The regulation of the thyroid-stimulating hormone of the anterior pituitary gland by thyroid hormone and by 9-cis-retinoic acid. Nutr Rev 2002;60:374–377. (*Current hypotheses on regulation of TSH by thyroid hormone*)

Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev 2000;81:1097–1142. (*Excellent current review of thyroid hormone action*)

Zhang J, Lazar M. The mechanism of action of thyroid hormones. Ann Rev Med 2000;62:439–466. (*Molecular dissection of thyroid hormone action*)

**Drug Summary Table** **Pharmacology of the Thyroid Gland**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **THYROID HORMONE REPLACEMENTS**<br>*Mechanism—Replace thyroid hormone* | | | | |
| **Levothyroxine (T4)** | Hypothyroidism | Hyperthyroidism | | T4 is used instead of T3 because of longer $t_{1/2}$ |
| **IODIDE UPTAKE INHIBITORS**<br>*Mechanism—Inhibit $Na^+/I^-$ symporter* | | | | |
| **Perchlorate**<br>**Thiocyanate**<br>**Pertechnetate** | Hyperthyroidism | Aplastic anemia | | Frequently used as radiocontrast agents |
| **ORGANIFICATION AND HORMONE RELEASE INHIBITORS**<br>*Mechanism—See Notes* | | | | |
| **$^{131}I^-$ (radioactive)** | Hyperthyroidism | May worsen ophthalmopathy in Graves' disease | Contraindicated in pregnancy | Excess radiation destroys thyroid, can cause hypothyroidism |
| **Iodide (high concentrations)** | Hyperthyroidism | May worsen toxic goiter symptoms | | Inhibits iodide uptake and organification (Wolff–Chaikoff effect) |
| **Propylthiouracil** | Hyperthyroidism | Lupus-like syndrome<br>Rare agranulocytosis<br>Vasculitis associated with antineutrophil cytoplasmic antibody (ANCA)<br>Hypoprothrombinemia | Preferred agent in pregnancy | Inhibits thyroid peroxidase<br>Inhibits T4 → T3 conversion |
| **Methimazole** | Hyperthyroidism | Lupus-like syndrome<br>Rare agranulocytosis | Contraindicated in pregnancy—may cause birth defects | Inhibits thyroid peroxidase<br>More potent than propylthiouracil |
| **INHIBITORS OF PERIPHERAL THYROID HORMONE METABOLISM**<br>*Mechanism—Block 5′-deiodinase* | | | | |
| **β-blockers** | Hyperthyroidism | ↓ Sympathetic activity | | Sympatholytic effect likely more important than effect on 5′-deiodinase |
| **Ipodate** | Hyperthyroidism | May exacerbate hyperthyroid symptoms | | Also used as radiocontrast agent |

## 26

# Pharmacology of the Adrenal Cortex

Ehrin J. Armstrong and Robert G. Dluhy

**Introduction**
**Case**
**Overview of the Adrenal Cortex**
**Glucocorticoids**
  Physiology
    *Synthesis*
    *Metabolism*
    *Physiologic Actions*
    *Regulation*
  Pathophysiology
    *Adrenal Insufficiency: Primary (Addison's Disease) and Secondary*
    *Glucocorticoid Excess: Cushing's Syndrome*
  Pharmacologic Classes and Agents
    *Cortisol and Glucocorticoid Analogues*
    *Inhibitors of Adrenocortical Hormone Synthesis*
    *Glucocorticoid Receptor Antagonists*
**Mineralocorticoids**
  Physiology
    *Synthesis*
    *Metabolism*
    *Physiologic Actions*
    *Regulation*
  Pathophysiology
    *Aldosterone Hypofunction*
    *Aldosterone Hyperfunction*
  Pharmacologic Classes and Agents
    *Mineralocorticoid Receptor Agonists*
    *Mineralocorticoid Receptor Antagonists*
**Adrenal Androgens**
  Physiology
  Pathophysiology
  Pharmacologic Classes and Agents
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

As with the pituitary gland, the adrenal gland consists of two organs fused together during embryologic development. The outer adrenal cortex originates from mesoderm, and the inner adrenal medulla is derived from neural crest cells. The adrenal cortex synthesizes and secretes steroid hormones essential for salt balance, intermediary metabolism, and androgenic actions in females. The adrenal medulla is important, although not essential, for maintaining sympathetic tone by means of secretion of the catecholamine epinephrine. This chapter focuses on the adrenal cortex; because of its importance in neuropharmacology, the adrenal medulla is discussed in Chapter 8, Adrenergic Pharmacology.

The pharmacologic utility of adrenocortical hormones spans almost every area of medicine. This is largely because of the use of glucocorticoid analogues as efficacious and potent anti-inflammatory agents. Unfortunately, long-term glucocorticoid therapy also produces a number of predictable but undesirable side effects. Inhibitors of biosynthetic enzymes in the adrenal cortex can be used to treat adrenocortical hormone excess. The physiology of mineralocorticoids is frequently manipulated in the treatment of hypertension, and there is much current interest in the development of mineralocorticoid receptor antagonists as a therapy for cardiovascular diseases. Finally, adrenal androgens, although lacking a definitive therapeutic indication, are frequently abused for their anabolic effects at high doses.

## Case

Johnny is 8 years old when he finds that he can barely catch his breath at times, especially while exercising. His asthma comes and goes, but no therapy seems to stop the asthma attacks completely. Although his doctor is concerned that it could stunt Johnny's growth, she eventually prescribes oral prednisone (a glucocorticoid analogue), and tells Johnny's parents to make sure he takes the medication every day. After a few weeks, Johnny's asthma attacks subside, and he is able to have a fairly normal childhood. During this time, the doctor pays close attention to Johnny's linear growth. Two years later, Johnny's doctor decides that a new inhaled glucocorticoid could be a safer medication for Johnny. Johnny switches to the inhaled glucocorticoid and discontinues oral prednisone. Three days later, he develops a respiratory infection and is brought to the emergency room with low blood pressure and a temperature of 103 degrees Fahrenheit. Based on his history of prednisone use, Johnny is immediately given hydrocortisone (cortisol) intravenously, as well as a saline infusion. Johnny recovers, and for the next 6 months slowly tapers his oral prednisone dose with continued use of the inhaled glucocorticoid. Eventually, he is able to take the inhaled glucocorticoid alone as an effective therapy for his asthma.

## QUESTIONS

1. Why are such cortisol analogues as prednisone used for treating asthma?
2. Why did the doctor monitor Johnny's linear growth?
3. Why did abrupt cessation of oral prednisone precipitate Johnny's clinical presentation in the emergency room?
4. Why are inhaled glucocorticoids safer than oral glucocorticoids for long-term treatment of asthma?

## OVERVIEW OF THE ADRENAL CORTEX

The adrenal cortex synthesizes three classes of hormones: **mineralocorticoids, glucocorticoids,** and **androgens.** Histologically, the adrenal cortex is divided into three zones. Moving from the capsule toward the medulla, these regions are the zona glomerulosa, zona fasciculata, and zona reticularis (Fig. 26-1). The glomerulosa is responsible for mineralocorticoid production and is under the control of **angiotensin II** and plasma **potassium** concentration. The fasciculata and reticularis synthesize glucocorticoids and androgens, respectively. Both the fasciculata and reticularis are under the control of **adrenocorticotropic hormone (ACTH),** which, in turn, is regulated by CRH and cortisol (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland).

Through its mineralocorticoid, glucocorticoid, and adrenal androgen products, the adrenal cortex plays a role in diverse aspects of homeostasis. The following discussion considers the physiology, pathophysiology, and pharmacology of each class of adrenal hormones. Because of their pharmacologic importance, the glucocorticoids are discussed first, followed by the mineralocorticoids and the adrenal androgens.

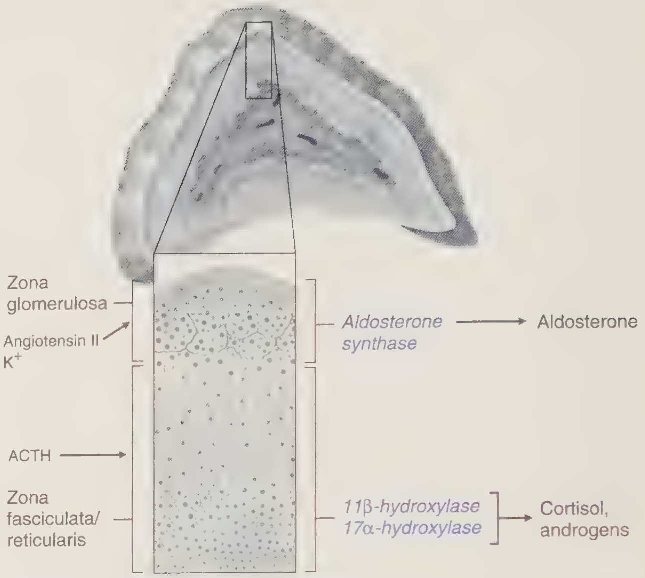


**Figure 26-1. Regions of the Adrenal Cortex.** The adrenal cortex is divided into three regions. The outermost region, the zona glomerulosa, synthesizes aldosterone and is regulated by circulating levels of angiotensin II and $K^+$. The zona fasciculata and zona reticularis synthesize cortisol and adrenal androgens. ACTH released from the anterior pituitary gland stimulates production of both cortisol and adrenal androgens. Tissue-specific expression of enzymes in each zone of the adrenal cortex—aldosterone synthase in the glomerulosa, steroid 11β-hydroxylase and steroid 17α-hydroxylase in the fasciculata/reticularis—determines the specificity of hormone production in that zone.

## GLUCOCORTICOIDS

### PHYSIOLOGY

### Synthesis

**Cortisol,** the endogenous glucocorticoid, is synthesized from cholesterol. Its synthesis begins with the rate-limiting conversion of cholesterol to pregnenolone, a reaction catalyzed by side-chain cleavage enzyme (Fig. 26-2). This first step converts the 27-carbon cholesterol into a 21-carbon precursor common to all adrenocortical hormones. From this precursor, steroid metabolism can proceed down three different pathways to generate mineralocorticoids, glucocorticoids, or adrenal androgens.

An oxidase enzyme catalyzes each step in the pathway of adrenocortical hormone synthesis. The oxidase enzymes are mitochondrial cytochromes, similar to the liver cytochrome **P450 oxidase system.** Tissue-specific expression of particular oxidase enzymes in each zone of the adrenal cortex provides the biochemical basis for the differences among the hormonal end products of the different zones of the cortex. For example, the zona fasciculata synthesizes cortisol, but not aldosterone or androgens (Fig. 26-1). This is because enzymes required uniquely for cortisol synthesis—such as steroid 11β-hydroxylase—are expressed in the zona fasciculata, whereas, enzymes required for aldosterone and androgen synthesis are not.

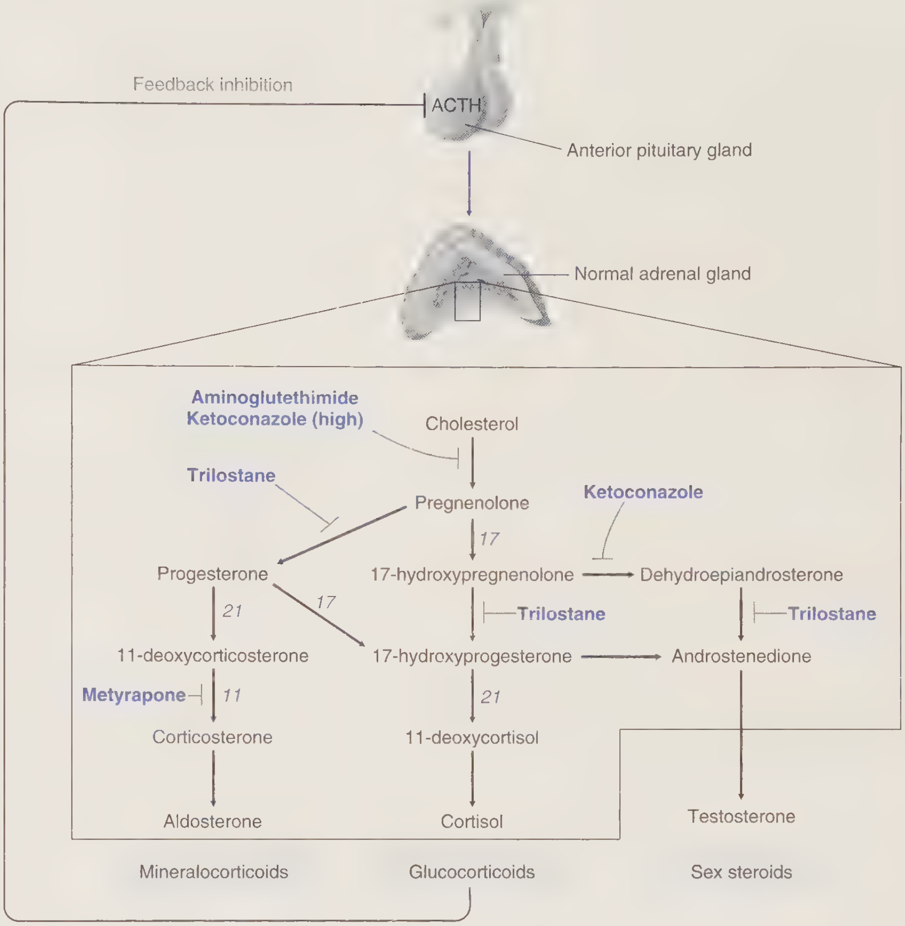

Feedback inhibition
ACTH
Anterior pituitary gland
Normal adrenal gland
Aminoglutethimide
Ketoconazole (high)
Cholesterol
Trilostane
Pregnenolone
Ketoconazole
17
Progesterone
17-hydroxypregnenolone
Dehydroepiandrosterone
21
17
Trilostane
Trilostane
11-deoxycorticosterone
17-hydroxyprogesterone
Androstenedione
Metyrapone
11
21
Corticosterone
11-deoxycortisol
Aldosterone
Cortisol
Testosterone
Mineralocorticoids
Glucocorticoids
Sex steroids

**Figure 26-2. Hormone Synthesis in the Adrenal Cortex.** The hormones of the adrenal cortex are steroids derived from cholesterol. The rate-limiting step in adrenal hormone biosynthesis is the modification of cholesterol to pregnenolone by side-chain cleavage enzyme. From this step, pregnenolone metabolism can be directed toward the formation of aldosterone, cortisol, or androstenedione. The flux of metabolites through each of these pathways depends on the tissue-specific expression of enzymes in the different cell types of the cortex and on the relative activity of the different synthetic enzymes. Note that several enzymes are involved in more than one pathway and that defects in these enzymes can affect the synthesis of more than one hormone. For example, a defect in steroid 21-hydroxylase prevents the synthesis of both aldosterone and cortisol. This overlap of synthetic activities also contributes to the nonselective action of glucocorticoid synthesis inhibitors; for example, trilostane. Enzymes are shown as numbers: 17, steroid 17α-hydroxylase; 21, steroid 21-hydroxylase; 11, steroid 11β-hydroxylase. Aminoglutethimide and high levels of ketoconazole inhibit side-chain cleavage enzyme. Ketoconazole primarily inhibits 17, 20-lyase. Trilostane inhibits 3β-hydroxysteroid dehydrogenase. Metyrapone inhibits steroid 11β-hydroxylase.

## Metabolism

Approximately 90% of circulating cortisol is bound to plasma proteins, the most important of which are **corticosteroid-binding globulin** (**CBG,** also referred to as transcortin) and **albumin.** CBG has high affinity for cortisol but low overall capacity, whereas albumin has low cortisol affinity but high overall capacity. Only molecules of cortisol that are unbound to protein (the so-called free fraction) are bioavailable, that is, available to diffuse through plasma membranes into cells. Thus, the affinity and capacity of plasma binding proteins regulate the availability of active hormone and, consequently, hormone activity.

The liver and kidneys are the primary sites of peripheral cortisol metabolism. Through reduction and subsequent conjugation to glucuronic acid, the liver is responsible for inactivating most of the cortisol in the plasma. This reaction also makes cortisol more water soluble, thus enabling renal excretion. Importantly, the liver and kidneys possess different isoforms of the enzyme **11β-hydroxysteroid dehydrogenase,** a regulator of cortisol activity. The two isoforms catalyze opposing reactions. In distal collecting duct cells of the kidney, 11β-hydroxysteroid dehydrogenase type II (11β-HSD II) converts cortisol to the biologically less active compound **cortisone,** which (unlike cortisol) does not bind to the mineralocorticoid receptor (see below, Fig. 26-3B). In contrast, cortisone can be converted back to cortisol (also referred to as hydrocortisone) in the liver by 11β-hydroxysteroid dehydrogenase type I (11β-HSDI, Fig. 26-3A). The interplay between these opposing reactions determines overall glucocorticoid activity. In addition, as discussed below, the activity of these enzymes is important in glucocorticoid pharmacology.

## Physiologic Actions

As with other steroid hormones, unbound cortisol diffuses through the plasma membrane into the cytosol of target cells, where the hormone then binds to a cytosolic receptor. There are two types of glucocorticoid receptors: the **Type I (mineralocorticoid)** and **Type II glucocorticoid receptors.** The Type I receptor is expressed in the organs of excretion (kidney, colon, salivary glands, sweat glands) and the hippocampus, whereas the Type II receptor has a broader tissue distribution. *The Type I glucocorticoid receptor is synonymous with the mineralocorticoid receptor.* The nomenclature is unfortunate, and this chapter hereafter refers to the Type I receptor as the "mineralocorticoid receptor."

Once cortisol binds to its cytosolic receptor and forms a hormone–receptor complex, the complex dimerizes with another hormone–receptor complex and is transported into the nucleus. In the case of cortisol, the dimerized hormone–

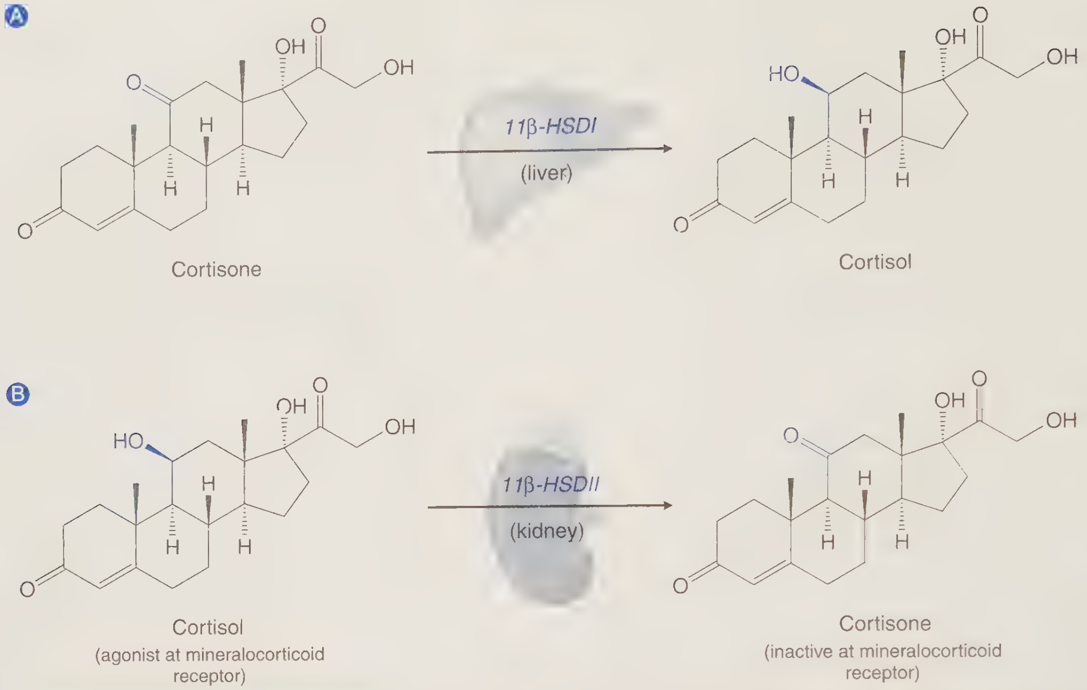

A
11β-HSDI
(liver)
Cortisone
Cortisol
B
11β-HSDII
(kidney)
Cortisol
(agonist at mineralocorticoid receptor)
Cortisone
(inactive at mineralocorticoid receptor)

**Figure 26-3. 11β-Hydroxysteroid Dehydrogenase.** The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) exists in two isoforms, which catalyze opposing reactions. **A,** In the liver, 11β-hydroxysteroid dehydrogenase type I (11β-HSDI) converts 11-keto glucocorticoids such as cortisone to 11-hydroxy glucocorticoids such as cortisol. **B,** In the kidney and other exocrine organs, 11β-hydroxysteroid dehydrogenase type II (11β-HSD II) converts cortisol to the inactive cortisone. This mechanism ensures that, at physiologic levels, cortisol does not exert mineralocorticoid effects. At high concentrations, however, cortisol (or another glucocorticoid) can overwhelm the activity of 11β-HSD II, leading to stimulation of the mineralocorticoid receptor as well as the glucocorticoid receptor.

receptor complex binds to gene promoter elements referred to as **glucocorticoid response elements (GREs),** which may either enhance or inhibit the expression of particular genes. Cortisol has profound effects on mRNA expression; about 10% of all human genes are estimated to contain GREs. Because of the large number of genes whose expression is affected by activation of GREs, cortisol has physiologic actions in most tissues. These actions can be divided generally into metabolic effects and anti-inflammatory effects.

*Metabolic effects of cortisol increase nutrient availability by raising blood glucose, amino acid, and triglyceride levels.* Cortisol increases blood glucose by antagonizing insulin action and by promoting gluconeogenesis in the fasting state. Cortisol also increases muscle protein catabolism, leading to increased levels of amino acids that can be utilized by the liver as fuels for gluconeogenesis. By potentiating growth hormone action on adipocytes, cortisol increases the activity of hormone-sensitive lipase and the subsequent release of free fatty acids (lipolysis). Cortisol levels increase as a component of stress responses induced by such events as acute trauma, surgery, fear, severe infection, and pain. By elevating blood glucose, the physiologic effects of glucocorticoids maintain energy homeostasis during the stress response, thus ensuring that such critical organs as the brain continue to receive nutrients.

In addition, cortisol has multiple anti-inflammatory actions. Cortisol negatively regulates cytokine release from cells of the immune system; this action may be an important mechanism to limit the extent of immune responses and to regulate the inflammatory response. In turn, certain cytokines, including IL-1, IL-2, IL-6, and TNF-α, can stimulate hypothalamic release of corticotropin releasing hormone, which stimulates ACTH and cortisol release. This series of stimulatory and inhibitory effects provides a feedback loop, in which inflammatory cytokines and cortisol are coordinately regulated to control immune and inflammatory responses (Fig. 26-4). Glucocorticoid-mediated suppression of the inflammatory response also has important pharmacologic implications for such clinical conditions as organ transplantation, rheumatoid arthritis, and asthma. Indeed, the introductory case demonstrates that glucocorticoids are an effective therapy for asthma. The exact mechanisms by which glucocorticoids act to ameliorate the symptoms of asthma are unknown, but are thought to be related to the ability of glucocorticoids to limit inflammation in the airways (see below and Chapter 45, Integrative Inflammation Pharmacology: Asthma).

## Regulation

The hypothalamic–pituitary unit coordinates the production of cortisol (refer to Chapter 24) for an overview. In response to central circadian rhythms and to stress, neurons from the paraventricular nucleus of the hypothalamus synthesize and secrete **corticotropin-releasing hormone (CRH),** a peptide hormone that travels through the hypothalamic–pituitary portal system. CRH then binds to G protein-coupled receptors on the

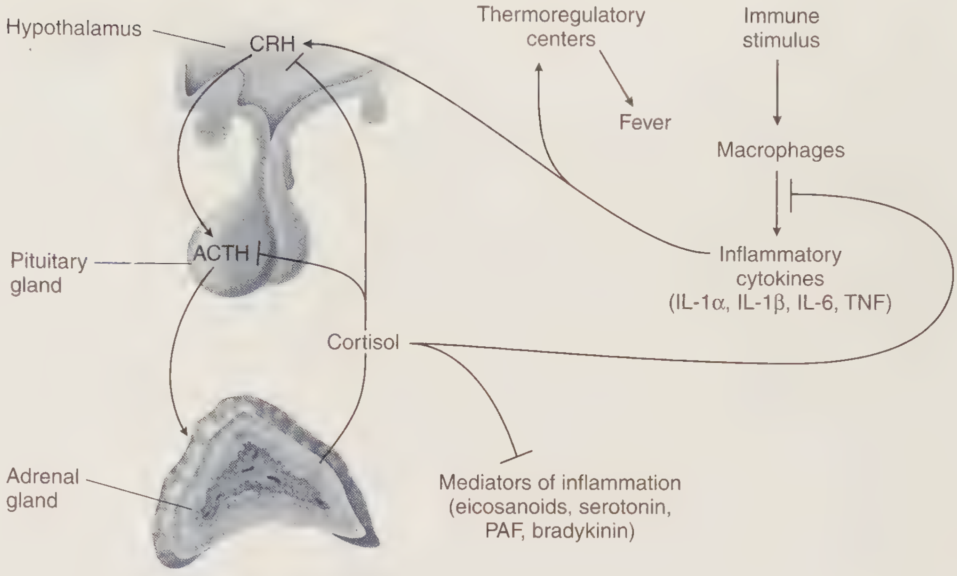

Hypothalamus
CRH
Thermoregulatory centers
Fever
Immune stimulus
Macrophages
Inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF)
Pituitary gland
ACTH
Cortisol
Adrenal gland
Mediators of inflammation (eicosanoids, serotonin, PAF, bradykinin)

**Figure 26–4. The Immune-Adrenal Axis.** Cortisol has profound immunosuppressive effects. Cortisol inhibits the action of several mediators of inflammation (eicosanoids, serotonin, platelet activating factor (PAF), bradykinin). Cortisol also inhibits the release of a number of cytokines from macrophages, including IL-1α, IL-1β, IL-6, and TNF-α. Because these cytokines promote the hypothalamic release of CRH and, thereby, increase serum levels of cortisol, it is hypothesized that the immune system uses the physiologic stress response to limit the extent of the inflammatory response.

surface of corticotroph cells in the anterior pituitary gland. CRH binding stimulates the corticotrophs to synthesize **proopiomelanocortin (POMC),** a precursor polypeptide that is cleaved into multiple peptide hormones including ACTH. Antidiuretic hormone, secreted by the posterior pituitary gland, synergizes with CRH to increase the release of ACTH by the anterior pituitary gland.

Proteolytic cleavage of POMC yields not only ACTH, but also γ-melanocyte–stimulating hormone (MSH), lipotropin, and β-endorphin. Melanocyte-stimulating hormone binds to receptors on skin melanocytes, promoting melanogenesis and, thereby, increasing skin pigmentation. Because of the similarities between the ACTH and MSH peptide sequences, high concentrations of ACTH can also bind to and activate MSH receptors. This action becomes apparent in primary hypoadrenalism (see below), in which increased ACTH levels result in increased skin pigmentation. The role of lipotropin in human physiology is uncertain, but is thought to involve control of lipolysis. β-Endorphin is an endogenous opioid that is important for pain modulation and for regulation of reproductive physiology.

Because steroid hormones are able to diffuse freely through cell membranes, and the adrenal gland stores little cortisol, ACTH regulates cortisol production at the synthesis stage. ACTH also has a trophic effect on the zona fasciculata and zona reticularis of the adrenal cortex, and hypertrophy of the cortex can occur in response to chronically elevated levels of ACTH.

As in other endocrine axes, there is negative feedback regulation by cortisol at the level of the hypothalamus and anterior pituitary gland. *High cortisol levels decrease both synthesis and release of CRH and ACTH.* Because ACTH has important trophic effects on the adrenal cortex, the absence of ACTH leads to atrophy of the cortisol-producing zona fasciculata and the androgen-producing zona reticularis. However, the aldosterone-producing zona glomerulosa cells continue to function, because angiotensin II and potassium are present to maintain production of aldosterone.

## PATHOPHYSIOLOGY

Diseases affecting glucocorticoid physiology can be divided into disorders of hormone deficiency and disorders of hormone excess. Addison's disease is the classic example of adrenocortical insufficiency, whereas Cushing's syndrome exemplifies cortisol excess.

### Adrenal Insufficiency: Primary (Addison's Disease) and Secondary

**Addison's disease** is an example of a primary adrenal insufficiency in which the adrenal cortex is selectively destroyed, most commonly by a T cell-mediated autoimmune reaction. Destruction of the cortex results in decreased synthesis of all classes of adrenocortical hormones. By comparison, secondary adrenal insufficiency is caused by hypothalamic or pituitary disorders or by prolonged administration of exogenous glucocorticoids. In secondary adrenal insufficiency, the decrease in ACTH levels causes decreased synthesis of sex hormones and cortisol but does not alter levels of aldosterone synthesis (see above).

Regardless of the underlying cause, adrenal insufficiency has serious consequences and can be life threatening if left untreated in the setting of stress. If the adrenal insufficiency is the result of high-dose, prolonged therapy with exogenous glucocorticoids, then the dose of glucocorticoid should be tapered slowly to allow the hypothalamic-pituitary–adrenal (HPA) axis to regain full activity. Importantly, it can take up to 1 year for the HPA axis to recover function following the abrupt discontinuation of exogenous glucocorticoid treatment.

In the introductory case, Johnny was switched from an oral glucocorticoid to an inhaled glucocorticoid that delivered a much lower systemic concentration of glucocorticoid. His adrenal cortex had atrophied because he had been maintained for 2 years on chronically high doses of prednisone, and he was, therefore, unable to produce a sufficient amount of cortisol in response to the stress of a respiratory infection. As a result, he arrived at the emergency room with acute adrenal insufficiency and required intravenous therapy with saline and hydrocortisone.

## Glucocorticoid Excess: Cushing's Syndrome

**Cushing's syndrome** refers to a number of underlying pathophysiologies, all of which increase cortisol production. The term "Cushing's disease" is reserved for ACTH-secreting pituitary adenomas that lead to increased cortisol production (Fig. 24-4C). Other causes of Cushing's syndrome include ectopic (inappropriate) secretion of ACTH, most commonly by small cell carcinomas of the lung (Fig. 24-4D) and, rarely, ectopic CRH syndrome. *Primary* Cushing's syndrome results from cortisol-secreting tumors (adenoma or carcinoma) of the adrenal cortex (Fig. 24-4B). However, iatrogenic Cushing's syndrome, secondary to pharmacologic treatment with exogenous glucocorticoids, is by far the most common cause of Cushing's syndrome.

The clinical features of Cushing's syndrome result from chronic overstimulation of target organs by endogenous or exogenous glucocorticoids. These features—which can include centripetal adipose redistribution, hypertension, proximal limb myopathy, osteoporosis, immunosuppression, and diabetes mellitus—reflect amplification of the normal physiologic actions of glucocorticoids in a variety of target tissues, and are discussed in more detail below.

# PHARMACOLOGIC CLASSES AND AGENTS

## Cortisol and Glucocorticoid Analogues

Drug therapy with glucocorticoids is indicated for two main purposes. First, exogenous glucocorticoids can be used as *replacement* hormone in cases of adrenal insufficiency. The goal of this therapy is to administer physiologic doses of glucocorticoids to ameliorate the effects of the adrenal insufficiency. Second, and more commonly, glucocorticoids are administered at *pharmacologic* doses to suppress inflammation and immune responses associated with such disorders as asthma, rheumatoid arthritis, and organ rejection following transplantation.

Because pharmacologic levels of systemic glucocorticoids invariably result in severe side effects, strategies to minimize these untoward responses to glucocorticoids have focused on local delivery of glucocorticoids to the area(s) requiring treatment. By limiting systemic exposure to the drug, HPA axis suppression and other features of iatrogenic Cushing's syndrome can be avoided. Examples of local glucocorticoid delivery include inhaled glucocorticoids for asthma, topical glucocorticoids for inflammatory skin conditions, and intra-articular glucocorticoids for rheumatoid arthritis.

A large number of glucocorticoid analogues have been synthesized. The following discussion highlights the differences among some commonly used cortisol analogues—including prednisone, prednisolone, fludrocortisone, and dexamethasone—by comparing the structures, potencies, and half-lives of these compounds to those of cortisol.

### *Structure and Potency*

Glucocorticoids can be divided into two classes based on the structural moiety present at the 11-carbon position. Compounds with a hydroxyl (–OH) group at the 11 position, such as cortisol, possess intrinsic glucocorticoid activity. In contrast, compounds with a carbonyl (=O) group at the 11 carbon, such as cortisone, are inactive until the liver enzyme 11β-hydroxysteroid dehydrogenase type I (11β-HSDI) reduces the compound to its 11-hydroxyl congener (Fig. 26-3). That is, cortisone is an inactive prodrug until it is converted by the liver to the active drug cortisol. *The native activity of a glucocorticoid is especially important for topically administered drugs because the skin does not possess appreciable amounts of 11β-HSDI.* Also, whenever possible, the active drug form is preferred over the inactive prodrug form for patients with liver dysfunction, because such patients may not be able to convert the prodrug to its active form.

All synthetic glucocorticoids are analogues of the endogenous glucocorticoid cortisol. The basic cortisol "backbone" is essential for glucocorticoid activity. As a consequence, all modifications consist of additions to this backbone (Fig. 26-5). For example, addition of a double bond between carbons 1 and 2 of cortisol creates **prednisolone** (Fig. 26-6), which has 4–5 times the anti-inflammatory potency of cortisol. Further addition of an α-methyl group (where α is defined as the side group orientation axial to the compound, while β is the equatorial orientation) to carbon 6 of prednisolone creates **methylprednisolone,** which has an anti-inflammatory potency 5–6 times that of cortisol.

Although prednisolone and methylprednisolone have significantly greater glucocorticoid potency than cortisol, addition of an α-fluorine (F) to carbon 9 of cortisol increases both the glucocorticoid and mineralocorticoid potencies of the resulting compound, known as **fludrocortisone.** Because of its enhanced mineralocorticoid activity, fludrocortisone is useful in the treatment of conditions characterized by mineralocorticoid deficiency (see below).

**Dexamethasone** incorporates two of the above changes to the cortisol backbone (1,2 double bond, 9α fluorine) as well as the addition of an α-methyl group at the 16-carbon position. This compound has more than 18 times the glucocorticoid potency of cortisol, but virtually no mineralocorticoid activity.

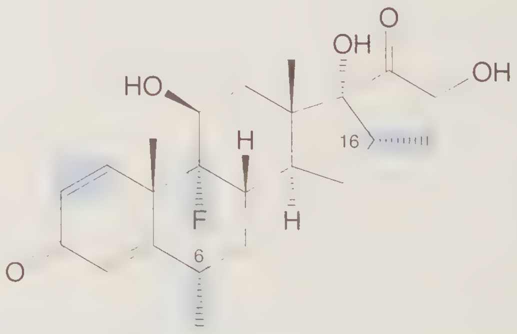


**Figure 26-5. Synthetic Modifications to the Cortisol Backbone.** Four modifications to the cortisol backbone are common in synthetic glucocorticoids. Addition of a 1-2 double bond (leftmost box), a methyl group at carbon 6, or a methyl group at carbon 16 increases the glucocorticoid activity of the compound relative to that of cortisol. Addition of a fluorine to carbon 9 increases glucocorticoid activity and markedly increases mineralocorticoid activity; the mineralcorticoid effect is blunted if 9-fluorination is combined with 16-methylation. Simultaneous addition of the 1-2 double bond, methyl at carbon 16, and fluorine at carbon 9 creates dexamethasone, which has very potent glucocorticoid activity but essentially no mineralocorticoid activity.

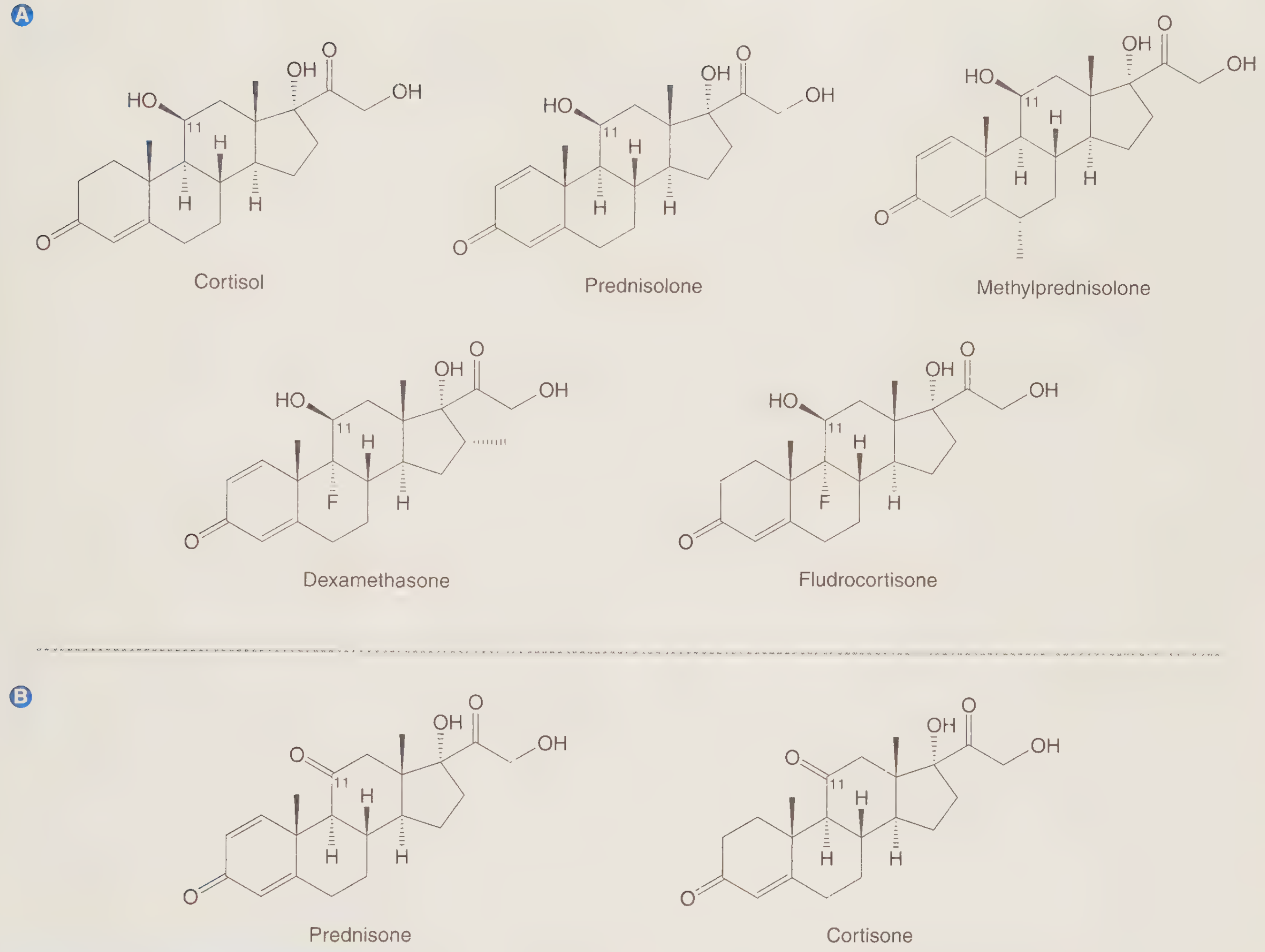


**Figure 26-6. Glucocorticoid Analogues.** Panel A shows a number of 11-hydroxy glucocorticoids, while panel B shows two 11-keto congeners. Note that the drugs in A are physiologically active, while the drugs in B are prodrugs that must be activated by 11β-HSDI to become active compounds. The structural class to which a glucocorticoid analogue belongs can be an important consideration in therapeutic decision making. For example, because the skin lacks significant 11β-HSDI activity, only 11-hydroxy glucocorticoids can be used in topical glucocorticoid creams, HSD, hydroxysteroid dehydrogenase.

A number of other permutations have been made to the cortisol backbone in other synthetic glucocorticoids, but the above discussion highlights the pertinent structural differences among the most common synthetic glucocorticoids. *Clinically, it is most important to be aware of the potency of each agent relative to cortisol, especially when considering a change from one analogue to another that has different relative glucocorticoid and mineralocorticoid activities.* Table 26-1 summarizes the relative glucocorticoid potencies and mineralocorticoid activities of several common glucocorticoid analogues.

## Duration of Action

The duration of glucocorticoid action is a complex pharmacokinetic variable that depends on:

1. Fraction of the drug bound to plasma proteins. More than 90% of circulating cortisol is protein-bound, primarily to CBG and, to a lesser degree, to albumin. In contrast, glucocorticoid analogues generally bind to CBG with relatively low affinity. As a result, approximately 2/3 of a typical glucocorticoid analogue circulates within plasma bound to albumin, while the rest is present as free steroid. Because only the free steroid is metabolized, the extent of binding to plasma proteins is a determinant of the drug's duration of action.
2. Affinity of the drug for 11β-HSD II. Glucocorticoids that have a lower affinity for 11β-HSD II have a longer plasma half-life because such drugs are not transformed into inactive metabolites as rapidly.
3. Lipophilicity of the drug. Increased lipophilicity promotes partitioning of the drug into adipose stores; the resulting decrease in the drug's metabolism and excretion extends its plasma half-life.
4. Affinity of the drug for the glucocorticoid receptor. Increased affinity of a drug for the glucocorticoid receptor increases the duration of action of a glucocorticoid analogue, because the fraction of drug that is bound to the receptor continues to exert its effect until the drug–receptor complex dissociates.

Together, these four variables result in a characteristic duration of action profile for each glucocorticoid analogue.

**TABLE 26-1** Relative Potencies and Durations of Action of Representative Glucocorticoid Analogues

| PHARMACOLOGIC AGENT | RELATIVE GLUCOCORTICOID POTENCY | RELATIVE MINERALOCORTICOID ACTIVITY | DURATION OF ACTION |
|---|---|---|---|
| Hydrocortisone | 1 | 1 | Short |
| Prednisolone | 4–5 | 0.25 | Short |
| Methylprednisolone | 5–6 | 0.25 | Short |
| Dexamethasone | 18 | < 0.01 | Long |

Short-acting agents have a tissue half-life of less than 12 hours, and long-acting agents have a half-life of greater than 48 hours.

Table 26-1 summarizes the duration of action of representative analogues as "short" or "long." *In general, glucocorticoid agents with higher anti-inflammatory (glucocorticoid) potency have a longer duration of action.*

### Replacement Therapy

Treatment of primary adrenal insufficiency is aimed at physiologically replacing both glucocorticoids and mineralocorticoids. **Oral hydrocortisone** is the glucocorticoid of choice. Because glucocorticoid replacement therapy must continue for life, the therapeutic goal is to administer the smallest possible effective dose of glucocorticoid so as to minimize the side effects of chronic glucocorticoid excess. Patients with primary adrenal insufficiency also require mineralocorticoid replacement, as described below. Patients with secondary adrenal insufficiency require only glucocorticoid replacement because, in this disorder, mineralocorticoid production is preserved by the renin–angiotensin system (see Chapter 19, Pharmacology of Volume Regulation).

### Pharmacologic Dosing

*Effects at Pharmacologic Levels.* Glucocorticoids are important mediators of the stress response, regulating both glucose homeostasis and the immune system. Glucocorticoids have found wide clinical use as anti-inflammatory agents because of their profound effect on immune and inflammatory processes. Pharmacologic levels of glucocorticoids inhibit **cytokine release** and, thereby, decrease IL-1, IL-2, IL-6, and TNF-α action. Local regulation of cytokine release is crucial for leukocyte recruitment and activation, and disruption of this signaling process profoundly inhibits immune function. Glucocorticoids also block the synthesis of arachidonic acid metabolites by inhibiting the action of phospholipase $A_2$. As discussed in Chapter 40, Pharmacology of Eicosanoids, arachidonic acid metabolites such as thromboxanes, prostaglandins, and leukotrienes mediate many of the early steps of inflammation, including vascular permeability, platelet aggregation, and vasoconstriction. By blocking the production of these metabolites, glucocorticoids significantly down-regulate the inflammatory response.

The multiple effects described above make glucocorticoids useful drugs in the treatment of a number of inflammatory and autoimmune diseases, such as asthma, rheumatoid arthritis, Crohn's disease, polyarteritis nodosa, temporal arteritis, and immune rejection following organ transplantation. It is important to note, however, that *pharmacologic glucocorticoid therapy does not correct the underlying disease etiology, but rather limits the effects of inflammation.* For that reason, discontinuing chronic glucocorticoid therapy often results in the resumption of inflammatory symptoms, unless the disorder has gone into a spontaneous remission.

Endogenous glucocorticoids affect many metabolic processes, and pharmacologic dosing with exogenous glucocorticoids amplifies those actions. Because of this, adverse effects typically accompany prolonged pharmacologic dosing. Increased **susceptibility to infection** is a potential side effect of long-term suppression of the inflammatory process by exogenous glucocorticoids. Glucocorticoids raise plasma **glucose levels** by antagonizing the action of insulin and promoting gluconeogenesis; pharmacologic doses of glucocorticoids amplify this effect. Insulin resistance and increased plasma glucose concentrations necessitate increased pancreatic β cell production of insulin to normalize blood glucose levels. As a result, **diabetes mellitus** is a common complication of long-term glucocorticoid administration, especially in patients with decreased pancreatic β cell reserve.

Pharmacologic dosing of glucocorticoids inhibits the vitamin D-mediated absorption of calcium. This results in **secondary hyperparathyroidism** and, therefore, an increase in bone resorption. Glucocorticoids also directly suppress osteoblast function. These two mechanisms contribute to bone loss, and long-term glucocorticoid therapy often results in **osteoporosis.** Steroid-induced bone resorption can be prevented with bisphosphonates, which inhibit osteoclast function and, thus, slow the progression of bone loss (see Chapter 29, Pharmacology of Bone Mineral Homeostasis). Chronic administration of glucocorticoids also slows **linear bone growth** in children. As a result, glucocorticoid administration can result in growth retardation and, ultimately, short stature in children who take glucocorticoids through adolescence. For this reason, Johnny's physician monitored Johnny's growth closely while he took oral prednisone.

Pharmacologic doses of glucocorticoids can cause selective atrophy of fast twitch muscle fibers, which results in muscle catabolism and weakness, primarily of the proximal muscles. Glucocorticoids also cause a characteristic redistribution of fat, with peripheral wasting of adipose stores but central obesity. Excessive fat deposition occurs on the back of the neck (buffalo hump) and face (moon facies).

In considering the potential for side effects of glucocorticoids, it is important to understand the concept of a population at risk. Not all individuals treated with glucocorticoids develop the same side effects because genetic and environmental variability places different individuals at risk for different sequelae of therapy. For example, a patient with borderline diabetes who is treated with glucocorticoids is likely to develop overt diabetes; whereas, a patient with sufficient pancreatic β cell reserve may not experience this side effect. *By carefully defining a patient's risk factors, it is often possible to forecast a particular patient's predisposition for side effects.*

*Withdrawal from Glucocorticoid Treatment.* A number of problems are often associated with the discontinuation of chronic glucocorticoid therapy. During long-term therapy with pharmacologic levels of glucocorticoids, high plasma glucocorticoid levels suppress the release of ACTH from the anterior pituitary gland and of CRH from the hypothalamus. Because ACTH has trophic effects on the adrenal cortex, the suppression of ACTH release during glucocorticoid therapy results in atrophy of the adrenal cortex. Abrupt cessation of glucocorticoid therapy can precipitate **acute adrenal insufficiency** because a number of months are required to reactivate the hypothalamic-pituitary–adrenal axis. Even after ACTH secretion recovers, a number of additional months may be required for the adrenal cortex to begin secreting physiologic levels of cortisol. Furthermore, the underlying inflammatory disease for which therapy was initiated can worsen during this time because of disinhibition of the immune system. Thus, it is axiomatic that *chronic glucocorticoid treatment should, whenever possible, be tapered slowly with gradually decreasing doses.* This taper allows the hypothalamus, anterior pituitary gland, and adrenal cortex to resume normal function gradually, thus avoiding adrenal insufficiency and, it is hoped, avoiding exacerbation of the underlying inflammatory condition.

Acute adrenal insufficiency occurred in the introductory case, because Johnny was switched acutely from oral prednisone to an inhaled glucocorticoid. On average, inhaled preparations deliver approximately 20% of the dose to the lung, while the other 80% is swallowed. However, the glucocorticoids available as inhaled formulations (see below) have significant first-pass hepatic metabolism, so that the swallowed portion is converted to inactive metabolites by the liver. Thus, Johnny's abrupt switch from an orally available glucocorticoid to an inhaled formulation caused acute adrenal insufficiency. After Johnny was placed back on oral prednisone, he was able to taper the dose slowly and, once his hypothalamic-pituitary–adrenal axis had been reactivated, use the inhaled glucocorticoid alone.

## Routes of Administration

Different drug delivery methods allow selective targeting of glucocorticoid to a particular tissue. The relevant concept is that it is possible to administer glucocorticoids locally at many times the normal plasma concentration, while minimizing untoward systemic side effects. Some examples of these methods include inhaled, cutaneous, and depot preparations of glucocorticoids. The administration of glucocorticoids during pregnancy is also an example of selective targeting because the placenta can metabolically partition glucocorticoids between mother and fetus (see below).

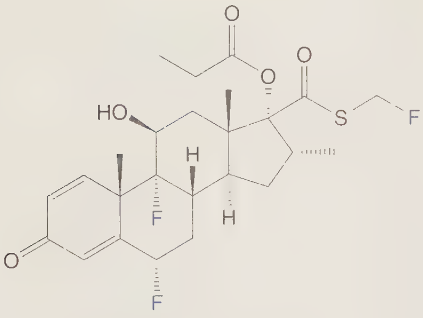
*Inhaled Glucocorticoids.* Inhaled glucocorticoids are the formulation of choice in the chronic treatment of asthma. Glucocorticoids reduce asthma symptoms by inhibiting airway inflammatory responses, especially eosinophil-mediated inflammation. The exact mechanism(s) is unknown but is thought to occur by inhibition of cytokine release and subsequent inhibition of the inflammatory cascade (see Chapter 45). Because systemic therapy with glucocorticoids can lead to many serious side effects, great efforts have been made to develop inhaled glucocorticoids with low oral bioavailability, thereby allowing high-dose delivery directly to the airway mucosa while minimizing systemic dosing. The goal of inhaled glucocorticoid therapy is to maximize the topical to systemic ratio of glucocorticoid concentration. This route of administration makes glucocorticoids safer for long-term dosing, especially when used in children such as Johnny.

Microcystalline powders and metered dose inhalers of glucocorticoids such as **fluticasone, beclomethasone, flunisolide, and triamcinolone** (Fig. 26-7) are currently available as inhaled formulations, allowing delivery of high concentrations of these potent glucocorticoids directly to the lung epithelium. The swallowed portion is absorbed into the portal circulation and, depending on the compound, hydroxylated by the liver to inactive metabolites. The significant first-pass metabolism of fluticasone, for example, ensures that less than 1% of the swallowed glucocorticoid is systemically bioavailable. *Thus, the systemic effects can be reduced by extensive first-pass hepatic metabolism of certain agents.* Although the portion of glucocorticoid delivered to the lung is eventually absorbed into the systemic circulation, the quantity delivered to the systemic circulation is lower than that for an oral glucocorticoid such as prednisone. Because the inhaled glucocorticoid is delivered directly to the inflamed organ, rather than via the systemic circulation, less inhaled glucocorticoid than oral glucocorticoid is required to control airway inflammation.

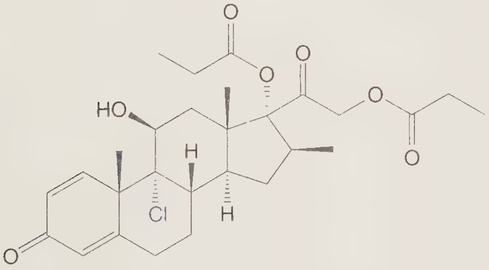
*If a patient treated chronically with systemic glucocorticoids is switched to inhaled glucocorticoids, care must be taken not to stop the systemic dosing abruptly.* As noted above, it is possible to precipitate acute adrenal insufficiency by suddenly switching systemic therapy to inhaled therapy, which provides a much lower systemic dose of glucocorticoid. Acute adrenal insufficiency can be life threatening and should be treated immediately with a large dose of intravenous glucocorticoid; for this reason, Johnny was given an intravenous infusion of prednisone in the introductory case.

**Oropharyngeal candidiasis** is another potential complication of inhaled glucocorticoid therapy because some glucocorticoid is delivered directly to the oral and pharyngeal mucosa. This results in local immunosuppression and allows infection with opportunistic organisms. Oropharyngeal candidiasis can be avoided by using mouthwash after each administration of aerosolized glucocorticoid.

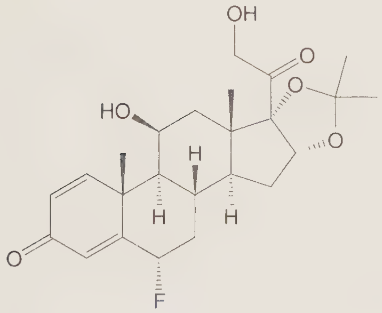
Many patients with asthma also have symptoms of allergic rhinitis. Intranasal administration of a glucocorticoid analogue is an effective therapy for these symptoms. This results in profound local suppression of the eosinophilic response



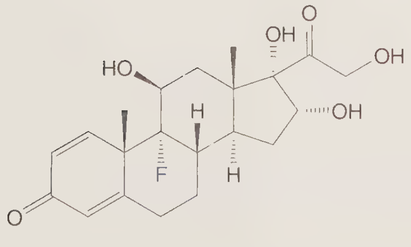
Fluticasone propionate



Beclomethasone dipropionate



Flunisolide



Triamcinolone

**Figure 26-7. Structures of Common Inhaled Glucocorticoids.** The currently available inhaled glucocorticoids are halogenated analogues of cortisol. These compounds are highly potent glucocorticoid agonists with little mineralocorticoid activity. Their high potency allows low doses of the inhaled glucocorticoids to inhibit the local inflammatory response that is a critical component of asthma pathophysiology. In addition, because a number of these compounds are subject to significant first-pass metabolism in the liver, the fraction of inhaled glucocorticoid that is inadvertently swallowed becomes inactivated before it can mediate undesirable systemic side effects.

and is often superior to antihistamines in the treatment of allergic rhinitis.

*Cutaneous Glucocorticoids.* Preparations of topical glucocorticoids are available for a number of dermatologic disorders, including psoriasis, lichen planus, and atopic dermatitis. Cutaneous administration delivers an extremely low percentage of the glucocorticoid systemically, allowing topical dosing at many-fold higher local concentrations than could be achieved safely with systemic administration. The glucocorticoid that is administered must be biologically active because the skin has little, if any, of the 11β-HSDI enzyme needed to convert glucocorticoid prodrugs to active compounds. Hydrocortisone, methylprednisolone, and dexamethasone are effective topical steroids for cutaneous use.

*Depot Glucocorticoids.* Depot intramuscular preparations of glucocorticoid analogues last for days to weeks and can be an alternative to daily or alternate-day oral glucocorticoids in the treatment of inflammatory diseases. Although depot formulations reduce the necessity for daily oral administration, these preparations are seldom used because the dose cannot be titrated on a frequent basis. Depot preparations of methylprednisolone suspended in polyethylene glycol are used, however, for **intra-articular administration.** This approach can be indicated for inflammatory processes restricted to the joints, such as rheumatoid arthritis or gout. Intra-articular glucocorticoid injection is useful in acute attacks of gout that are unresponsive to colchicine or indomethacin. Intra-articular and bursae injection requires the use of active glucocorticoid, because joint tissue lacks the 11β-HSDI enzyme required to hydroxylate and, thus, activate 11-keto glucocorticoids.

*Pregnancy.* The placental–maternal barrier provides another example of selective glucocorticoid targeting. During pregnancy, the placenta metabolically separates the fetus from the mother. Because of this, prednisone can be administered to the mother during pregnancy without fetal side effects. The maternal liver activates the prednisone to prednisolone, but fetal placental 11β-HSD II is capable of converting the prednisolone back to the inactive prednisone. Because the liver does not function during fetal life, the fetus does not, in turn, activate prednisone. *Therefore, use of the "prodrug" prednisone in pregnancy does not result in delivery of an active glucocorticoid to the fetus.*

Glucocorticoids also promote lung development in the fetus. If glucocorticoid therapy is indicated to promote lung maturation in a fetus, dexamethasone is commonly administered to the mother. Dexamethasone is a poor substrate for placental 11β-HSD II, and is, therefore, present in active form in the fetal circulation, where it stimulates maturation of the lung. The dose must be titrated carefully because exposure to excessive glucocorticoid can have a number of deleterious effects on fetal development.

## Inhibitors of Adrenocortical Hormone Synthesis

A number of compounds are available to inhibit hormone biosynthesis by the adrenal cortex. Although these drugs have some specificity for individual adrenal enzymes (Table 26-2), it is not typically possible to alter the production of a single adrenal hormone independent of other hormones. Because the enzymes necessary for adrenal hormone synthesis are P450

**TABLE 26-2** Sites of Action and Pathways Affected by Inhibitors of Adrenal Hormone Synthesis

| INHIBITOR | SITE OF ACTION | ADRENAL STEROIDOGENIC PATHWAYS AFFECTED |
|---|---|---|
| Mitotane | Mitochondria | All |
| Aminoglutethimide | Side-chain cleavage enzyme | All (aromatase also inhibited in ovary) |
| Ketoconazole | Primarily 17, 20-lyase | Low concentrations: ↓ Androgen synthesis<br>High concentrations: All (gonadal steroid hormones also inhibited) |
| Metyrapone | 11β-hydroxylase | Cortisol synthesis |
| Trilostane | 3β-hydroxysteroid dehydrogenase | Cortisol and aldosterone synthesis |

enzymes, the use of these inhibitors is also associated with potential toxicity to hepatic P450 enzymes. In general, these agents can be divided into drugs that affect earlier versus later steps in adrenal hormone synthesis. The agents that inhibit early steps have broad effects, while those affecting later steps have more selective actions. Mitotane, aminoglutethimide, and ketoconazole inhibit early steps in adrenal hormone synthesis.

**Mitotane** is a structural analogue of DDT that is toxic to adrenocortical mitochondria. Although used infrequently, mitotane may be indicated for medical adrenalectomy in cases of severe Cushing's disease or in adrenocortical carcinoma. Patients taking mitotane commonly develop hypercholesterolemia because of concomitant inhibition of cholesterol oxidase by the drug.

**Aminoglutethimide** inhibits side-chain cleavage enzyme. Aminoglutethimide has also been shown to inhibit the enzyme aromatase, which is important for conversion of androgens to estrogens. The ability of aminoglutethimide to inhibit aromatase is under investigation as a potential therapy for breast cancer (see Chapter 27, Pharmacology of Reproduction).

**Ketoconazole** is an antifungal agent that is typically used to inhibit the activity of the fungal P450 enzymes (see Chapter 36, Pharmacology of Fungal Infections). Because the enzymes that mediate adrenal and gonadal hormone synthesis are also members of the P450 enzyme family, high doses of ketoconazole also suppress steroid synthesis in these organs. This agent primarily inhibits the action of 17, 20-lyase (important for adrenal androgen synthesis). High doses of ketoconazole also suppress the activity of side-chain cleavage enzyme, the enzyme that converts cholesterol to pregnenolone. Because the generation of pregnenolone is common to the synthesis of all adrenal hormones, high-dose ketoconazole inhibits the synthesis of a number of steroid compounds.

Metyrapone and trilostane have more specific effects on adrenal hormone synthesis. **Metyrapone** inhibits 11β-hydroxylation, resulting in impaired cortisol synthesis (Fig. 26-2). Because cortisol is the adrenal steroid responsible for feedback inhibition of ACTH release, treatment with metyrapone also results in disinhibition of ACTH secretion. Thus, metyrapone can be administered as a test of ACTH reserve.

**Trilostane** is a reversible inhibitor of 3β-hydroxysteroid dehydrogenase. Thus, administration of this agent leads to reduced aldosterone and cortisol production in the adrenal cortex.

### Glucocorticoid Receptor Antagonists

**Mifepristone** (RU-486) is a progesterone receptor antagonist used to induce abortion early in pregnancy (see Chapter 27). At higher concentrations, mifepristone also blocks the glucocorticoid receptor. This action makes mifepristone potentially useful for the treatment of life-threatening elevated glucocorticoid levels, such as in ectopic ACTH syndrome, although its clinical usefulness for this purpose has not yet been evaluated fully.

# MINERALOCORTICOIDS

## PHYSIOLOGY

### Synthesis

Like cortisol, **aldosterone** is a 21-carbon steroid hormone derived from cholesterol. Enzymes unique to aldosterone synthesis are expressed only in the zona glomerulosa, and are under the regulation of the renin–angiotensin system and potassium.

### Metabolism

Circulating aldosterone binds with low affinity to transcortin, albumin, and a specific mineralocorticoid binding protein. Approximately 50%–60% of circulating aldosterone is bound to transport proteins, resulting in a short elimination half-life (20 minutes). Orally administered aldosterone also has a high first-pass hepatic metabolism, with approximately 75% of the hormone metabolized to an inactive form during each pass through the liver. As a result, aldosterone cannot be administered orally as effective replacement therapy in adrenal insufficiency states.

### Physiologic Actions

Mineralocorticoids play important roles in regulating sodium reabsorption in sweat and salivary glands, the colon, and the kidney. In each of these organs, circulating aldosterone diffuses through the plasma membrane and binds to a cytosolic **mineralocorticoid receptor** (synonymous with the Type I glucocorticoid receptor). The aldosterone:mineralocorticoid receptor complex is then transported into the nucleus, where it binds to mineralocorticoid response elements present on specific gene promoters and, thus, up-regulates or down-regulates gene expression. Studies have also demonstrated rapid nongenomic actions of aldosterone, which may be mediated by hormone binding to a cell surface aldosterone receptor. The physiologic role of this second signaling mechanism is currently unknown.

A major role of aldosterone is to increase **$Na^+$/$K^+$ATPase** expression in the basolateral membrane of distal nephron cells. Enhanced $Na^+$/$K^+$ ATPase activity secondarily increases sodium reabsorption and potassium secretion across the lumenal epithelium of the nephron (see Chapter 19). As a result, sodium retention, potassium excretion, and $H^+$ excretion are all enhanced by aldosterone. Excess aldosterone can cause hypokalemic alkalosis, while hypoaldosteronism can cause hyperkalemic acidosis.

Although aldosterone has classically been considered important for sodium homeostasis and potassium regulation, recent data also support extrarenal actions of aldosterone in cardiovascular tissues. Animal studies have demonstrated **aldosterone-mediated cardiac fibrosis,** but only in the setting of salt loading. The fibrosis appears to be a reparative process secondary to inflammatory necrosis. Importantly, antagonists of aldosterone action at the mineralcorticoid receptor, such as spironolactone, may be useful pharmacologic agents for preventing these cardiac actions.

### Regulation

Three systems regulate aldosterone synthesis: the renin-angiotensin–aldosterone system, plasma potassium levels, and ACTH.

The **renin-angiotensin–aldosterone system** is a central regulator of extracellular fluid volume. Decreases in extracellular fluid volume decrease perfusion pressure at the afferent arteriole of the renal glomerulus, which acts as a baroreceptor. This stimulates the juxtaglomerular cells to secrete renin, a protease that cleaves the prohormone angiotensinogen to angiotensin I. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme, which is expressed at high concentrations by the capillary endothelium of the lungs. Angiotensin II has direct arteriolar pressor effects, and it stimulates aldosterone synthesis by binding to and activating a G protein-coupled receptor in zona glomerulosa cells of the adrenal cortex.

**Potassium loading** increases aldosterone synthesis independent of renin activity. Because aldosterone activity at the distal nephron increases potassium excretion, this control mechanism serves a homeostatic role in regulating potassium balance.

Finally, **ACTH** acutely stimulates aldosterone synthesis in the zona glomerulosa. ACTH is believed to play only a minor physiologic role in aldosterone synthesis, however. Unlike cortisol, aldosterone does not negatively regulate ACTH secretion.

## PATHOPHYSIOLOGY

### Aldosterone Hypofunction

Aldosterone hypofunction (hypoaldosteronism) can result from a primary decrease in aldosterone synthesis or action, or from a secondary decrease in aldosterone regulators, such as renin. Most cases of hypoaldosteronism result from decreased aldosterone synthesis. Defects in the gene CYP21, which encodes an oxidase necessary for both aldosterone and glucocorticoid synthesis, lead to congenital adrenal hyperplasia (discussed under adrenal androgen pathophysiology) and cause salt wasting as a result of aldosterone deficiency. **Addison's disease,** or primary adrenal insufficiency, results in hypoaldosteronism secondary to destruction of the zona glomerulosa. Most cases of Addison's disease are caused by autoimmune adrenalitis; other causes include tuberculosis and metastatic cancer. In each case, aldosterone hypofunction can lead to salt wasting, hyperkalemia, and acidosis. Finally, hypoaldosteronism can result from states of decreased renin production (so-called hyporeninemic hypoaldosteronism, which is common in diabetic renal insufficiency), from resistance to the action of aldosterone at the level of the mineralocorticoid receptor, or from inactivating mutations of the aldosterone-regulated epithelial sodium channel (ENaC) in the cortical collecting duct of the nephron.

### Aldosterone Hyperfunction

**Primary hyperaldosteronism** results from excess aldosterone production by the adrenal cortex. Bilateral zona glomerulosa adrenal hyperplasia or an aldosterone-producing adenoma are the most common causes. Increased aldosterone synthesis leads to positive sodium balance, with consequent extracellular volume expansion, suppression of plasma renin activity, potassium wasting and hypokalemia, and hypertension.

## PHARMACOLOGIC CLASSES AND AGENTS

### Mineralocorticoid Receptor Agonists

Pathophysiologic conditions leading to hypoaldosteronism necessitate replacement with physiologic doses of a mineralocorticoid. It is not possible to administer aldosterone itself as a therapeutic agent, because the liver converts over 75% of oral aldosterone to an inactive metabolite during first-pass metabolism. Instead, the cortisol analogue **fludrocortisone,** which has minimal first-pass hepatic metabolism and a high mineralocorticoid to glucocorticoid potency ratio, is used. The side effects of fludrocortisone therapy are all related to the ability of this agent to mimic a state of mineralocorticoid excess, including hypertension, hypokalemia, and even cardiac failure. It is, therefore, crucial to monitor serum potassium and blood pressure levels closely in all patients receiving fludrocortisone therapy to ensure that an appropriate dose of drug is being administered.

### Mineralocorticoid Receptor Antagonists

**Spironolactone** (also discussed in Chapters 19 and 27) is a competitive antagonist at the mineralocorticoid receptor, but the drug also binds to and inhibits the androgen and progesterone receptors. The latter actions, which result in such side effects as gynecomastia in males, limit the usefulness of this agent in some patient subsets. **Eplerenone** is a mineralocorticoid receptor antagonist that binds only to the mineralocorticoid receptor; this specificity may make eplerenone free of the unwanted side effects of spironolactone. Both spironolactone and eplerenone can be used as antihypertensive agents, and both are approved for use in patients with heart failure.

## ADRENAL ANDROGENS

### PHYSIOLOGY

Sex steroids produced by the adrenal cortex, primarily **dehydroepiandrosterone** (DHEA), have an uncertain role in human physiology. DHEA seems to be a prohormone that is converted to more potent androgens, primarily testosterone, in the periphery. Adrenocortical androgens are an important source of testosterone in females; these hormones are necessary for the development of female axillary and pubic hair at the time of puberty, when adrenal androgen secretion is activated (adrenarche).

### PATHOPHYSIOLOGY

**Congenital adrenal hyperplasia** (CAH) and **polycystic ovarian syndrome** are two important diseases related to adrenocortical androgen production. Congenital adrenal hyperplasia is a clinical term denoting a number of inherited enzyme deficiencies in the adrenal cortex. These abnormalities cause hirsutism and virilization in females as a result of increased adrenocortical androgen production. Polycystic ovarian syndrome, discussed in Chapter 27, may be caused by congenital adrenal hyperplasia in a subset of patients.

The most common form of congenital adrenal hyperplasia results from a deficiency of **steroid 21-hydroxylase.** Deficiency of 21-hydroxylase results in the inability of adrenal cortex cells to synthesize both aldosterone and cortisol (Fig. 26-8). Because cortisol is the main negative feedback regulator of pituitary ACTH release, the decreased cortisol synthesis that results from 21-hydroxylase deficiency disinhibits ACTH release. Increased ACTH restores the level of cortisol, but there is also shunting of precursor compounds into the "unblocked" androgen pathway, resulting in greater production of DHEA and androstenedione. The liver subsequently converts these compounds into testosterone. In severe 21-hydroxylase deficiency, there may be a virilizing effect on the developing female fetus. As a result, female neonates with 21-hydroxylase deficiency typically have masculinized or ambiguous exter-

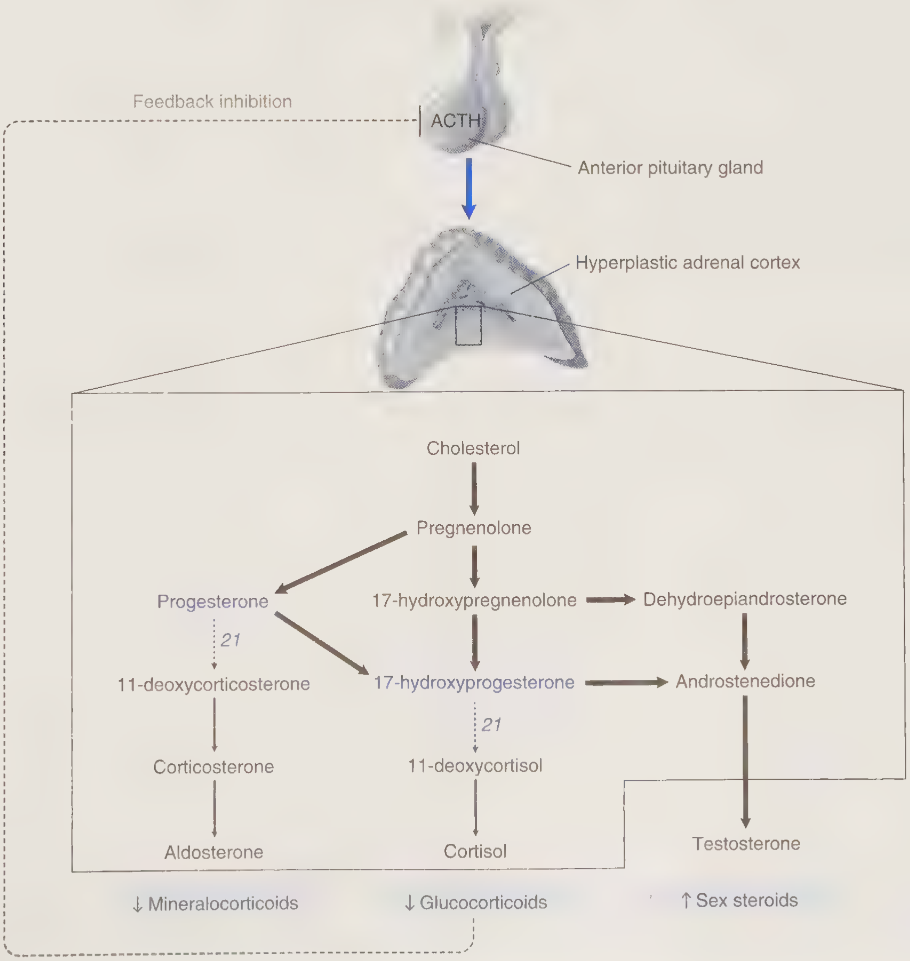

Feedback inhibition
ACTH
Anterior pituitary gland
Hyperplastic adrenal cortex
Cholesterol
Pregnenolone
Progesterone
17-hydroxypregnenolone
Dehydroepiandrosterone
21
11-deoxycorticosterone
17-hydroxyprogesterone
Androstenedione
21
Corticosterone
11-deoxycortisol
Aldosterone
Cortisol
Testosterone
↓ Mineralocorticoids
↓ Glucocorticoids
↑ Sex steroids

**Figure 26-8. Congenital Adrenal Hyperplasia.** Steroid 21-hydroxylase deficiency, the most common cause of congenital adrenal hyperplasia, results in impaired biosynthesis of aldosterone and cortisol (dashed lines). Therefore, steroid hormone synthesis in the adrenal cortex is shunted toward increased production of sex steroids (thick lines). The lack of cortisol production decreases the negative feedback on corticotroph cells of the anterior pituitary gland (dashed line), causing increased ACTH release (thick blue arrow). Increased levels of ACTH induce adrenal hyperplasia, and further stimulate the synthesis of sex steroids. This pathway can be interrupted by administering exogenous cortisol. The deficient enzyme is shown as a number: 21, steroid 21-hydroxylase.

nal genitalia. In the male, however, increased adrenal androgens may have little or no noticeable phenotypic effect. Instead, males with 21-hydroxylase deficiency are commonly diagnosed in infancy during an acute salt-wasting crisis, which results from the inability to synthesize aldosterone. Mild 21-hydroxylase deficiency may manifest later in life as hirsutism, acne, and oligomenorrhea in young women after menarche.

Treatment of congenital adrenal hyperplasia is aimed at glucocorticoid replacement, which suppresses excessive hypothalamic and pituitary release of CRH and ACTH, resulting in decreased production of adrenal androgens.

## PHARMACOLOGIC CLASSES AND AGENTS

The androgens synthesized by the adrenal gland can be viewed as prohormones. Because no specific receptors for either DHEA or androstenedione have been described, the activity of these hormones depends on conversion of the hormones to testosterone, and subsequently to dihydrotestosterone, in peripheral target tissues. As discussed above, adrenal androgen excess can cause a variety of syndromes in women; the pharmacologic interruption of excess androgenic activity is discussed in Chapter 27.

DHEA is not regulated by the FDA and is commonly used as an "over the counter" drug. The indications for its use are understudied, however. Population cross-sectional studies have shown a reciprocal relationship between an age-related decline in DHEA levels and the risk of cardiovascular disease and cancer. Replacement therapy with DHEA may be indicated for cases of Addison's disease in which there is bona fide DHEA deficiency. Some studies have reported decreased DHEA levels in chronic fatigue syndrome, and although studies are currently underway to determine the efficacy (if any) of DHEA treatment in this syndrome, DHEA is commonly used to treat chronic fatigue.

Exogenous DHEA can be converted to testosterone in the testes. As a result, DHEA is commonly abused for its anabolic effects.

## *Conclusion and Future Directions*

Aldosterone, cortisol, and the adrenal androgens regulate many aspects of basic homeostasis. Aldosterone regulates extracellular fluid volume by promoting sodium reabsorption and fluid retention. Cortisol regulates diverse physiologic processes, including energy homeostasis and inflammatory responses. The physiologic role of adrenal androgens is unknown, but pathophysiologic states causing increased adrenal androgen production have significant masculinizing effects in women. Antagonists of aldosterone are currently used as diuretics, and recent evidence supports an additional role for these agents in the prevention of cardiac fibrosis. To this end, antagonists specific for the aldosterone receptor may become important therapies for heart disease. Glucocorticoid pharmacology is an immense field, primarily because glucocorticoids are used to suppress inflammation in a number of disease states. Chronic glucocorticoid use is associated with a multitude of predictable side effects, and future research in this area will attempt to minimize the side effects of glucocorticoid therapy while maintaining the anti-inflammatory actions. Such efforts could include the development of tissue-specific or selective glucocorticoid agonists and antagonists (analogous to the selective estrogen receptor modulators), as well as further refinement of drug delivery methods. Finally, the pharmacology of adrenal androgens needs to be studied more extensively to determine the indications, if any, for DHEA therapy.

## *Suggested Readings*

Barnes PJ. Inhaled glucocorticoids for asthma. N Engl J Med 1995; 332:868–875. (*Good discussion of glucocorticoid kinetics*)

Barnes PJ, Adcock IM. How do corticosteroids work in asthma? Ann Intern Med 2003;139:359–370. (*Current review of pathophysiology and treatment of inflammation in asthma*)

Kroboth PD, Salek FS, Pittenger AL. DHEA and DHEA-S: A review. J Clin Pharmacol 1999;39:327–348. (*Succinct review of pertinent physiology and pharmacology*)

Oelkers W. Adrenal insufficiency. N Engl J Med 1996;335: 1206–1212. (*Pathophysiology and treatment of adrenal insufficiency*)

Sapolsky R. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Rev 2000;21:55–89. (*Thorough review discussing the numerous roles of glucocorticoids in stress responses*)

Williams JS, Williams GH. 50th anniversary of aldosterone. J Clin Endocrinol Metab 2003;88:2364–2372. (*Current review of mineralocorticoids*)

## Drug Summary Table Pharmacology of the Adrenal Gland

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **GLUCOCORTICOID RECEPTOR AGONISTS**<br>*Mechanism—Mimic cortisol function as agonists at the glucocorticoid receptor* | | | | |
| **Cortisol**<br>**Cortisone**<br>**Prednisone**<br>**Prednisolone**<br>**Methylprednisolone**<br>**Dexamethasone** | Inflammatory states<br>Autoimmune disease | Immunosuppression<br>Cataracts<br>Increased glucose levels<br>Osteoporosis<br>Growth retardation<br>Muscle atrophy | Abrupt withdrawal leads to acute adrenal insufficiency | See Table 26-1 for relative potency and duration of action of individual agents<br>Cortisol (hydrocortisone) is used as replacement therapy in primary and secondary adrenal insufficiency |
| **Fluticasone**<br>**Beclomethasone**<br>**Flunisolide**<br>**Triamcinolone** | Asthma, allergic rhinitis | Oropharyngeal candidiasis | Do not switch abruptly from oral to inhaled agents | Intranasal and inhaled formulations that greatly reduce systemic side effects |
| **INHIBITORS OF GLUCOCORTICOID SYNTHESIS**<br>*Mechanism—Inhibit various steps in hormone synthesis* | | | | |
| **Mitotane** | Adrenocortical carcinoma<br>Severe Cushing's syndrome | CNS depression, nausea<br>Hypercholesterolemia | | Toxic to mitochondria of adrenal cortex<br>Inhibits peripheral metabolism of cortisol |
| **Aminoglutethimide** | Cushing's syndrome | Pruritus | | ↓ Levels of all adrenal cortex hormones |
| **Ketoconazole** | Cushing's syndrome | Gynecomastia | | Preferentially inhibits sex steroid synthesis |
| **Metyrapone** | Test of ACTH reserve | | | Preferentially inhibits cortisol synthesis |
| **Trilostane** | Cushing's syndrome | Diarrhea, nausea | | Decreases synthesis of both aldosterone and cortisol |
| **GLUCOCORTICOID RECEPTOR ANTAGONISTS**<br>*Mechanism—Competitive antagonist of cortisol action at the glucocorticoid receptor* | | | | |
| **Mifepristone** | Cushing's syndrome | Hypothyroidism | | Also an antiprogestin; main clinical use is medical abortion |
| **MINERALOCORTICOID RECEPTOR AGONISTS**<br>*Mechanism—Agonist at the mineralocorticoid receptor* | | | | |
| **Fludrocortisone** | Hypoaldosteronism | Hypertension, hypokalemia | | |
| **MINERALOCORTICOID RECEPTOR ANTAGONISTS**<br>*Mechanism—Competitive antagonist of aldosterone action at the mineralocorticoid receptor* | | | | |
| **Spironolactone** | Diuretic<br>Hypertension<br>Congestive heart failure | Gynecomastia<br>Hyperkalemia<br>Metabolic acidosis | Renal failure | May be cardioprotective; also antagonizes androgen receptor |
| **Eplerenone** | Diuretic<br>Hypertension<br>Congestive heart failure | Hyperkalemia<br>Metabolic acidosis | Renal failure | Selective for mineralocorticoid receptor |
| **ADRENAL SEX STEROID**<br>*Mechanism—Unknown, converted to androgen* | | | | |
| **DHEA** | None approved | | | Often used in chronic fatigue syndrome, hypoadrenalism, substance abuse |

# 27

# Pharmacology of Reproduction

Ehrin J. Armstrong and Daniel D. Federman

**Introduction**
**Case**
**Physiology of Reproductive Hormones**
  Synthesis of Progestins, Androgens, and Estrogens
  Hormone Action and Metabolism
  Hypothalamic-Pituitary–Reproductive Axis
  Integration of Endocrine Control: The Menstrual Cycle
**Pathophysiology**
  Disruption of the Hypothalamic-Pituitary–Reproductive Axis
  Inappropriate Growth of Hormone-Dependent Tissues
  Decreased Estrogen or Androgen Secretion
**Pharmacologic Classes and Agents**
  Inhibitors of Gonadal Hormones
    *Synthesis inhibitors*
    *Receptor Antagonists*
  Hormones and Hormone Analogues: Contraception
    *Combination Oral Contraception*
    *Morning-After Contraception*
    *Progestin-Only Contraception*
    *Male Contraception*
  Hormones and Hormone Analogues: Replacement
    *Estrogens*
    *Androgens*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

This chapter presents endocrine pharmacology relevant to both the male and female reproductive tracts. Although men and women differ in their hormonal profiles, androgens and estrogens are both under the control of anterior pituitary gland gonadotropins and, ultimately, hypothalamic release of gonadotropin-releasing hormone (GnRH). Female hormone patterns are more complex than male patterns: Hormonal control of the menstrual cycle is an illustrative example of how sex hormones are integrated into a complex physiologic system. Understanding the menstrual cycle also provides a basis for understanding the pharmacology of contraception. A number of diseases are treated pharmacologically via modification of reproductive hormone activity; these range from infertility and endometriosis to breast and prostate cancer. Key concepts in this chapter include: (1) the interactions between estrogen and the pituitary gland; (2) the effects of GnRH release frequency on gonadotropin release; (3) the tissue selectivity of estrogen receptor agonists and antagonists; and (4) the various strategies used to antagonize the effects of endogenous sex hormones, from suppression of the hypothalamic-pituitary–reproductive axis to antagonism at the target tissue receptor. Because of its historical role in the prevention of osteoporosis, estrogen replacement therapy is discussed both here and in Chapter 29, Pharmacology of Bone Mineral Homeostasis. Androgen replacement therapy is discussed at the end of this chapter.

## Case

Amy Johnston first notices that her hair is thinning somewhat during her teenage years. Even though she loses some hair on her scalp, Ms. Johnston notices excessive hair growth on her face; she sometimes has to shave to remove inappropriate hair growth. At age 27, she goes to her doctor complaining of both her hair problem and the fact that her periods are irregular. Upon further questioning, the doctor discovers that the longest interval between her menstrual cycles has been 6 months and the shortest 22 days. When Ms. Johnston does have periods, they are heavy and last for more than her previous average of 5 days. The increased hair growth on her face, extremities, abdomen, and breasts had begun around age 15. Ms. Johnston also reports a problem with being overweight since finishing college, where she had been extremely active in soccer, field hockey, and swimming. The doctor orders several tests, and finds that Ms. Johnston has mildly elevated free and total testosterone levels, normal estradiol but increased estrone levels, and an increased ratio of plasma luteinizing hormone to follicle stimulating hormone.

Based on these findings, the doctor tells Ms. Johnston that she probably has a disorder called polycystic ovarian syndrome. He prescribes spironolactone to reduce her problems with hair growth and balding. He also recommends combination oral contraceptives to regularize her menstrual cycles.

## QUESTIONS

- **1.** What is the pathophysiologic link between excessive hair growth and infertility?
- **2.** Why was spironolactone prescribed to reduce Ms. Johnston's hair problem?
- **3.** How do oral contraceptives act, and how would they help regulate Ms. Johnston's menstrual cycles?

## PHYSIOLOGY OF REPRODUCTIVE HORMONES

### SYNTHESIS OF PROGESTINS, ANDROGENS, AND ESTROGENS

The synthesis of progestins, androgens, and estrogens is closely intertwined. All three groups are steroid hormones derived from the metabolism of cholesterol. The synthesis of these hormones is similar to that of adrenal sex hormones, which is discussed in Chapter 26, Pharmacology of the Adrenal Cortex.

The terminology "progestins," "androgens," and "estrogens" denotes a number of related hormones, rather than a single molecule in each group (Fig. 27-1). The **progestins** consist of **progesterone,** a common precursor to estrogen and testosterone synthesis (see also Fig. 26-2), and a number of synthetically altered progesterone derivatives used for therapeutic purposes. Progestins generally exert antiproliferative effects on the female endometrium by promoting the endometrial lining to secrete rather than proliferate (see below). Progesterone is also required for the maintenance of pregnancy. **Androgens,** all of which have masculinizing properties, include dehydroepiandrosterone (DHEA), androstenedione, **testosterone,** and **dihydrotestosterone,** among which testosterone is considered the classic androgen. Androgens are required for conversion to a male phenotype during development and for male sexual maturation. **Estrogens** refer to a number of substances that share a common feminizing activity. **17β-Estradiol** is the most potent naturally occurring estrogen, while estrone and estriol are less potent.

Note that estrogen synthesis depends on androgen precursors; in general, *estrogens are aromatized forms of the androgen substances from which they are derived* (Fig. 27-1). Although most of this interconversion occurs in the testes or ovaries, a small but significant amount of sex hormone synthesis also occurs in the zona reticularis of the adrenal cortex. The action of adrenal sex hormones is most notable in postmenopausal women; after menopause, the majority of circulating estrogen is derived from adipose tissue because fat cells contain the **aromatase** enzyme necessary for converting androgens to estrogens. This is also the main source of circulating estrogens in men. Importantly, however, the adrenal gland is able to make the weak androgen DHEA, but is not able to synthesize testosterone. As a result, the estrogens of postmenopausal women consist primarily of estrone rather than the more potent estradiol.

### HORMONE ACTION AND METABOLISM

Progestins, androgens, and estrogens are all hormones that bind to a related family of receptors; glucocorticoids, mineralocorticoids, vitamin D, and thyroid hormone also bind to the same superfamily of receptors. Once synthesized, these hormones diffuse into the bloodstream, where they bind tightly to such carrier proteins as sex-hormone binding globulin and albumin. Only the unbound fraction of hormone is able to diffuse into cells and bind to an intracellular receptor. Interestingly, *testosterone is essentially a prohormone.* Testosterone binds to the androgen receptor, but with only modest affinity. As a result, testosterone has only modest androgenic activity. Instead, testosterone is converted in target tissues to the more active **dihydrotestosterone** (Fig. 27-2), which binds to the androgen receptor with ten times higher affinity than does testosterone. The formation of dihydrotestosterone from testosterone is catalyzed by the enzyme **5α-reductase.** There are at least two subtypes of 5α-reductase. Differential tissue expression of these enzymes provides some pharmacologic specificity for the 5α-reductase inhibitors. The importance of dihydrotestosterone as the most active androgen is highlighted in individuals with inherited deficiencies of 5α-reductase. Males lacking this enzyme are phenotypically female, because they are unable to convert testosterone to dihydrotestosterone and are, thus, unable to activate a program of male differentiation during development.

Although separate progesterone, androgen, and estrogen receptors exist, complete selectivity of action does not exist because of the close structural similarities of the hormones. For example, most progestins have significant cross reactivity with androgen receptors, and prolonged progestin administration produces an androgenic effect (**virilization,** or the development of masculine features). Most synthetic progestins used for contraception and hormone replacement therapy have been modified to minimize their androgenic side effects.

The **estrogen receptor** (ER) is the best studied of the sex hormone receptors and serves as an example for all three receptor types. Because progestins, androgens, and estrogens are lipophilic steroid hormones, the fraction of hormone that remains unbound to plasma proteins can freely diffuse across the plasma membrane into the cytosol of cells. Once inside the cell, the hormone ligand binds to its specific intracellular receptor, which subsequently dimerizes. For example, association of estrogen with the estrogen receptor causes dimerization of two estrogen–estrogen receptor complexes, and the dimer then binds to **estrogen response elements** (EREs) present in promoter regions of DNA. This binding to EREs, together with the recruitment of co-activators or co-repressors, enhances or inhibits the transcription of specific genes and, thereby, causes the physiologic effects of the hormone.

There are two subtypes of estrogen receptors—ERα and ERβ. In addition, it is now recognized that many estrogen receptor actions involve association of the receptor with other transcription cofactors; that is, that dimerization of the estrogen receptor and subsequent binding of the dimer to EREs are insufficient to explain the complex and varied actions of es-

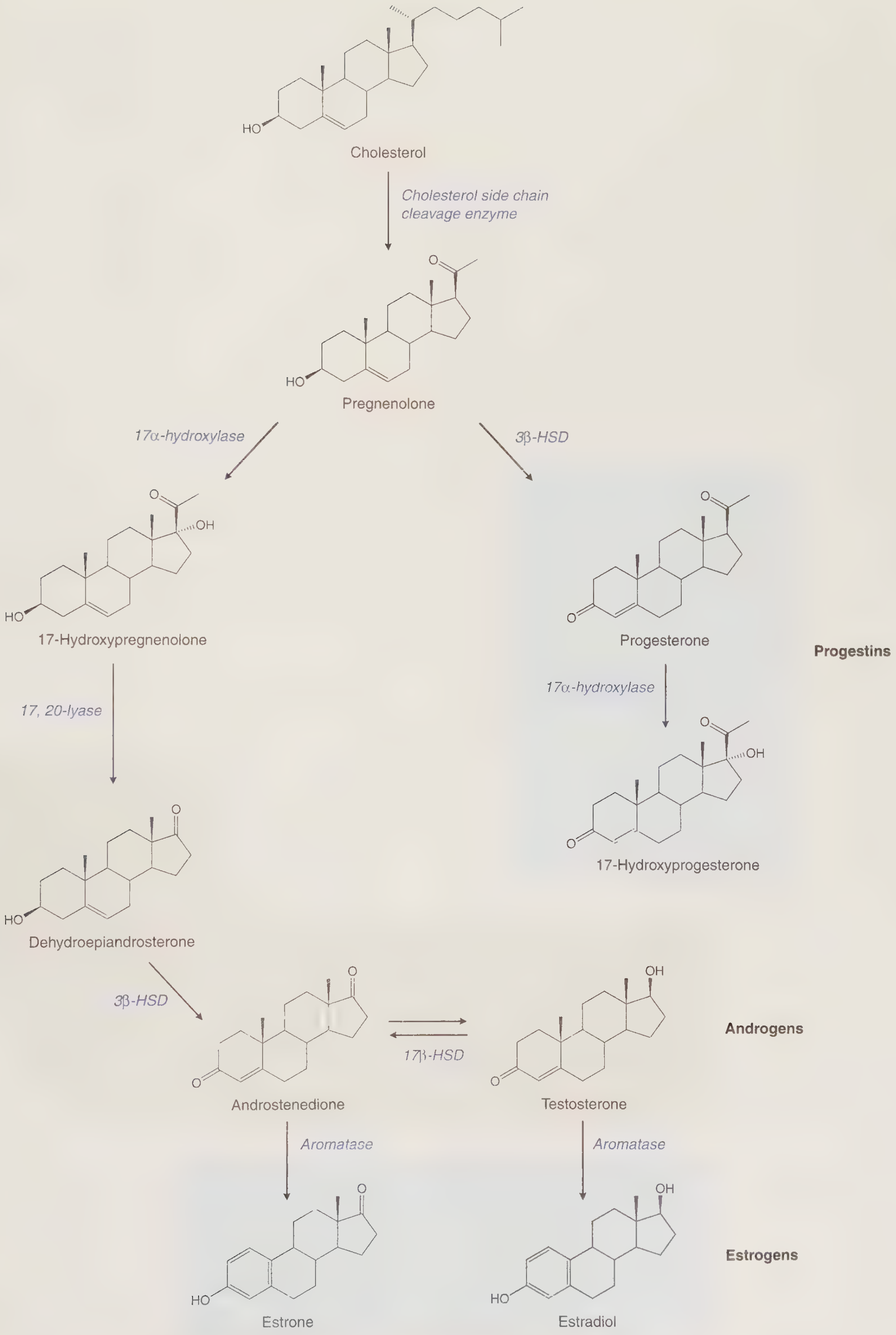

HO
Cholesterol
Cholesterol side chain cleavage enzyme
O
HO
Pregnenolone
17α-hydroxylase
3β-HSD
O
OH
HO
17-Hydroxypregnenolone
O
O
Progesterone
Progestins
17α-hydroxylase
O
OH
O
17-Hydroxyprogesterone
17, 20-lyase
O
HO
Dehydroepiandrosterone
3β-HSD
O
O
Androstenedione
17β-HSD
OH
O
Testosterone
Androgens
Aromatase
Aromatase
O
HO
Estrone
OH
HO
Estradiol
Estrogens

**Figure 27-1. Synthesis of Estrogens, Progestins, and Androgens.** Estrogens, progestins, and androgens are steroid hormones derived from cholesterol. The major progestins include progesterone and 17α-hydroxyprogesterone. The androgens include dehydroepiandrosterone (DHEA), androstenedione, and testosterone. Estrogens include estrone, and estradiol. Estrogens are aromatized forms of their conjugate androgens: androstenedione is aromatized to estrone, and testosterone is aromatized to estradiol. Estradiol and estrone are both metabolized to estriol, a weak estrogen (not shown). HSD, hydroxysteroid dehydrogenase.

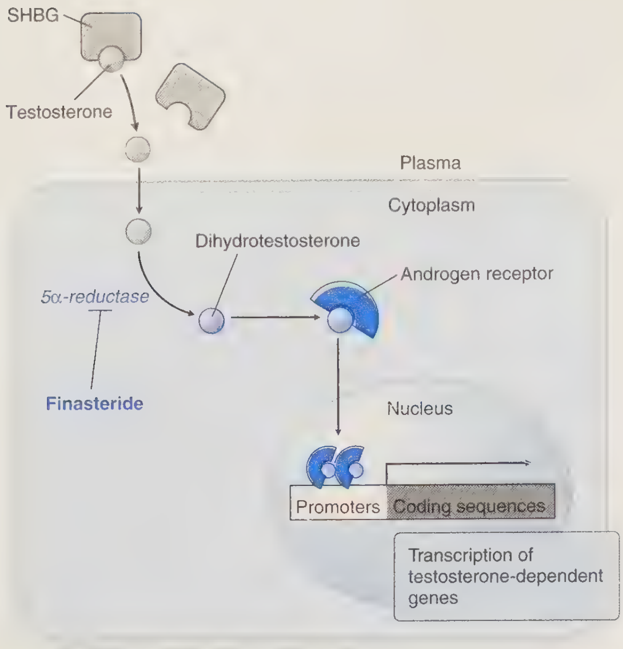


**Figure 27-2. Peripheral Conversion of Testosterone.** Testosterone circulates in the plasma bound to sex hormone binding globulin (SHBG) and/or albumin (not shown). Free testosterone diffuses through the plasma membrane of cells into the cytosol. In target tissues, the enzyme 5α-reductase converts testosterone to dihydrotestosterone, which has increased androgenic activity relative to testosterone. Dihydrotestosterone binds with high affinity to the androgen receptor, forming a complex that is transported into the nucleus. Homodimers of dihydrotestosterone and androgen receptor initiate transcription of testosterone-dependent genes. Finasteride, a drug used in the treatment of benign prostatic hypertrophy and male pattern hair loss, inhibits the enzyme 5α-reductase.

trogen in different tissues. *The specific transcription factors that are recruited by the estrogen receptor seem to be tissue-dependent, and probably account for some of the target specificity of estrogen action.* Although the subtypes and molecular associations of the androgen and progesterone receptors have not been studied as thoroughly as those of the estrogen receptor, it is likely that the same complexities exist for these receptors. The recognition that differential binding of modular transcription factors to ERs could alter estrogenic effects will likely prove a burgeoning area of pharmacologic research in the near future, as pharmaceutical researchers continue to develop receptor agonists and antagonists with selective actions on specific tissues. Selective estrogen receptor modulators (SERMs, see below) are the first drugs to take advantage of the tissue selectivity of sex hormone receptor function.

## HYPOTHALAMIC-PITUITARY–REPRODUCTIVE AXIS

A common hypothalamic-pituitary–gonadal axis regulates sex hormone synthesis. Gonadotropin releasing hormone (GnRH) resides at the top of this three-tiered hierarchy. The hypothalamus secretes GnRH in pulses (Fig. 27-3). GnRH travels via the hypothalamic–pituitary portal system to stimulate gonadotroph cells of the anterior pituitary gland. Stimulation of gonadotroph cells via a G protein-coupled cell surface receptor increases the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are jointly referred to as the gonadotropins.

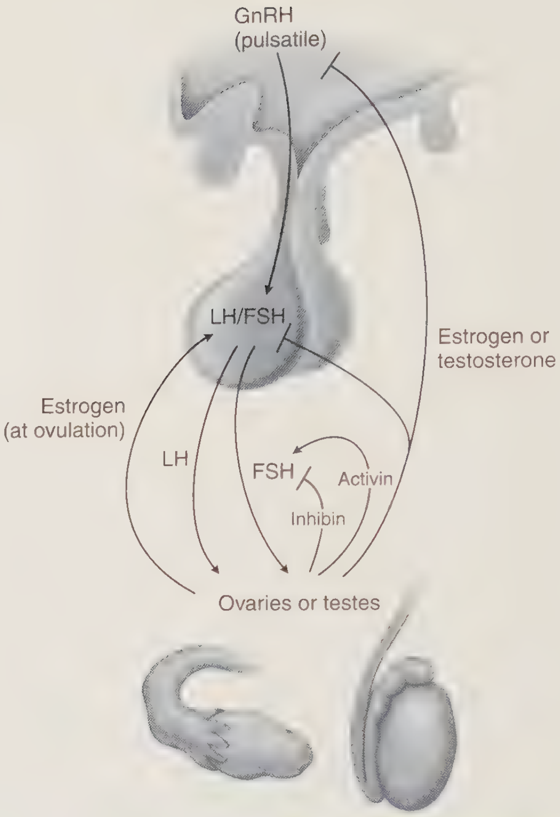


**Figure 27-3. The Hypothalamic-Pituitary–Reproductive Axis.** The hypothalamus secretes gonadotropin releasing hormone (GnRH) into the hypothalamic-pituitary portal system in a pulsatile pattern. GnRH stimulates gonadotroph cells in the anterior pituitary gland to synthesize and release luteinizing hormone (LH) and follicle stimulating hormone (FSH). These two hormones, referred to as gonadotropins, promote ovarian and testicular synthesis of estrogen and testosterone, respectively. Estrogen and testosterone inhibit release of GnRH, LH, and FSH. Depending on the time in the menstrual cycle, the concentration of estrogen in the plasma, and the rate at which estrogen concentration increases in the plasma, estrogen can also stimulate pituitary gonadotropin release (e.g., at ovulation). Both the ovaries and testes secrete inhibin, which selectively inhibits FSH secretion, and activin, which selectively promotes FSH secretion.

Although one cell type produces both LH and FSH, the synthesis and release of these two hormones are controlled independently. Current research suggests that the rate of GnRH secretion may preferentially alter the secretion patterns of LH and FSH. Pulsatile secretion of GnRH is critical for the proper functioning of the hypothalamic-pituitary–reproductive axis. *When GnRH is administered continuously, gonadotroph release of LH and FSH is suppressed rather than stimulated.* This effect has the important pharmacologic consequence that pulsatile administration of exogenous GnRH stimulates gonadotropin release, whereas continuous GnRH administration inhibits LH and FSH release and, therefore, blocks target cell function.

LH and FSH have analogous but slightly different effects in males and females. The pertinent target cells in the male are the **Leydig** and **Sertoli** cells of the testis, while the **thecal** and **granulosa** cells of the ovary mediate gonadotropin function in the female (Fig. 27-4). In each case, a two-cell system is coordinated to mediate sex hormone actions. LH stimulates either testicular Leydig cells or ovarian thecal cells to increase the synthesis of androgen, which then diffuses into neighboring Sertoli or granulosa cells, respectively. In the male, Leydig cells synthesize the androgen testosterone; in the female, thecal cells synthesize the androgen androstenedione, which is then converted to testosterone in the granulosa cells. Both the Sertoli and granulosa cells aromatize testosterone to estrogen. In addition, both Sertoli and granulosa cells express FSH receptors. In the Sertoli cell, FSH stimulation increases the production of androgen binding protein, which is important for maintaining the high testicular concentrations of testosterone necessary for spermatogenesis. In addition, FSH stimulates the Sertoli cell to produce other proteins necessary for sperm maturation. In the granulosa cell, FSH stimulates aromatase activity, thus increasing the conversion of testosterone to estrogen.

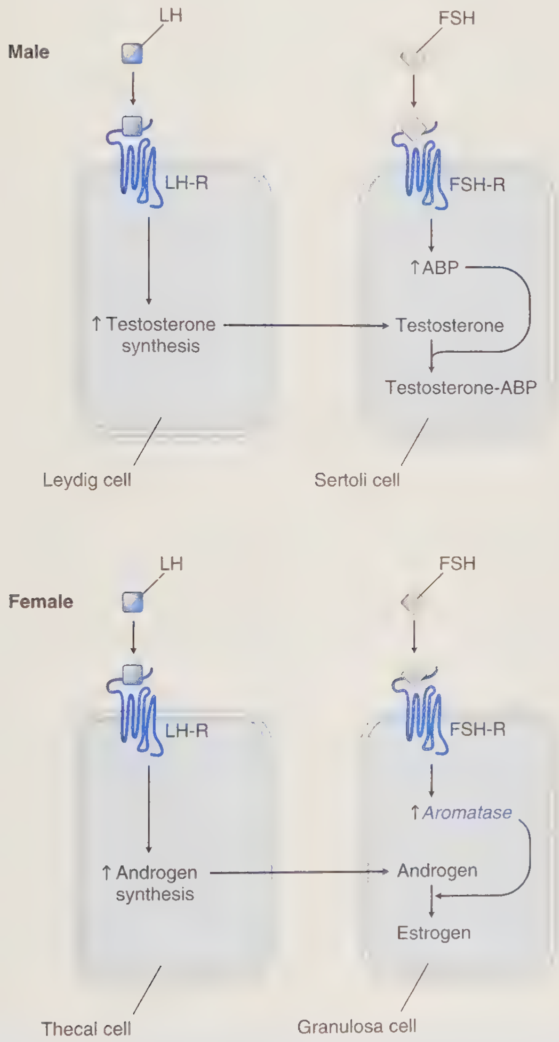


**Figure 27-4. Two-Cell Systems for Gonadal Hormone Action.** In the male, the binding of luteinizing hormone (LH) to the LH receptor (LH-R) activates testosterone synthesis in Leydig cells. Testosterone then diffuses into nearby Sertoli cells, where the binding of follicle stimulating hormone (FSH) to its receptor (FSH-R) increases levels of androgen binding protein (ABP). ABP stabilizes the high concentrations of testosterone that, together with other FSH-induced proteins synthesized in Sertoli cells, promote spermatogenesis in the nearby germinal epithelium (not shown). In the female, LH acts in an analogous manner to promote androgen (androstenedione) synthesis in thecal cells. Androgen then diffuses into nearby granulosa cells, where (after the androstenedione is converted to testosterone; not shown), aromatase converts testosterone to estrogen. FSH increases aromatase activity in granulosa cells, promoting the conversion of androgen to estrogen. Note that dihydrotestosterone is not a substrate for aromatase.

Both Sertoli cells and granulosa cells synthesize and secrete the regulatory proteins **inhibin** and **activin.** Inhibin feedback inhibits pituitary gonadotroph release of FSH, while activin stimulates FSH release. Neither inhibin nor activin has an effect on pituitary gland LH release (Fig. 27-3). The role of these regulatory proteins in controlling hormone action is still not completely understood. In the male, testosterone is also an important negative regulator of pituitary gland and hypothalamic hormone release. The role of estrogen in the female is more complex, and can involve either positive or negative feedback depending on the prevailing hormonal milieu; this topic is addressed below as part of the menstrual cycle discussion.

## INTEGRATION OF ENDOCRINE CONTROL: THE MENSTRUAL CYCLE

The female menstrual cycle is governed by the cycling of hormones with an approximate periodicity of 28 days. This cycle begins at the onset of puberty and continues uninterrupted (with the exception of pregnancy) until menopause (Fig. 27-5). The start of the cycle is arbitrarily defined as the onset of menstruation. Ovulation occurs at the midportion (about day 14) of each cycle. The portion of the menstrual cycle before ovulation is often referred to as either the **follicular** or **proliferative** phase because, during this time, the developing ovarian follicle produces most of the gonadal hormones, which stimulate proliferation of the endometrium. Subsequent to ovulation, the **corpus luteum** produces progesterone, and the endometrium becomes secretory rather than proliferative. The second half of the menstrual cycle is, thus, often referred to as the **luteal** or **secretory** phase, depending on whether the producer or the target of sex hormone action is considered as the frame of reference.

At the start of the menstrual cycle, there is low production of estrogen. As a result, the anterior pituitary gland secretes increasing amounts of FSH and LH. These hormones stimulate the maturation of 6–8 follicles, each of which contains an ovum arrested in the first stage of meiosis. Maturing follicles secrete increasing concentrations of estrogen. Estrogen causes the follicles to increase the expression of LH and FSH receptors on thecal and granulosa cells, respectively. Receptor upregulation increases the follicular response to pituitary gland gonadotropins and allows one follicle to secrete increasing quantities of estrogen. The increased plasma estrogen levels partially suppress pituitary gland LH and FSH release. In turn, the decreased gonadotropin levels cause other follicles

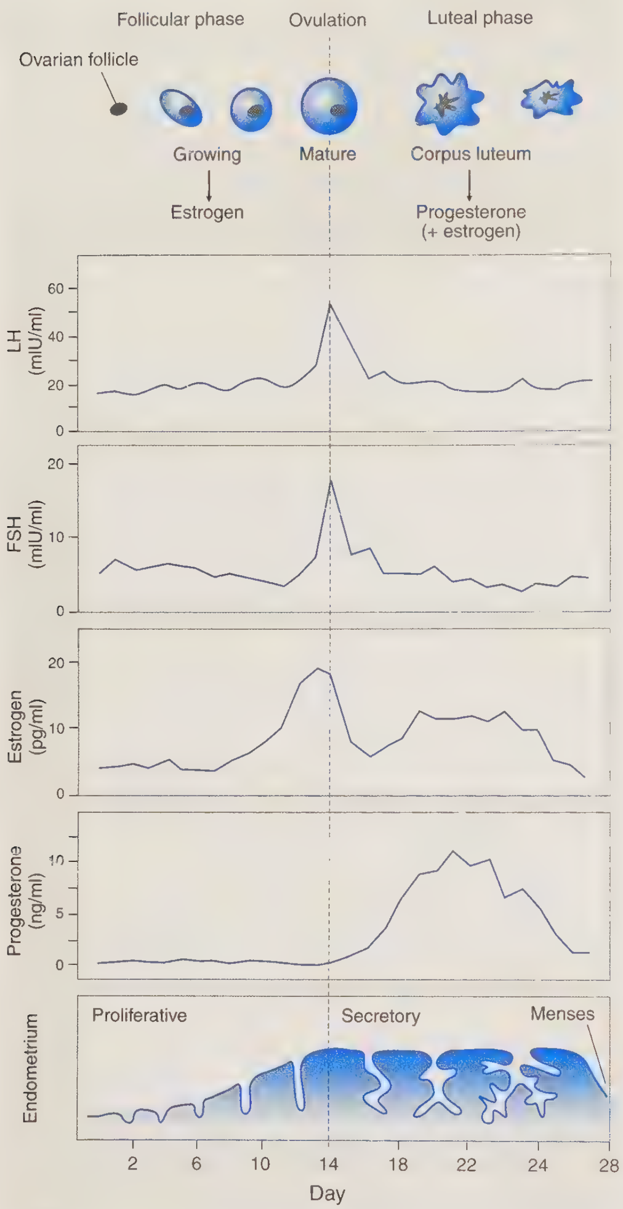


**Figure 27-5. The Menstrual Cycle.** The menstrual cycle is divided into the follicular phase and the luteal phase. Ovulation defines the transition between these two phases. During the follicular phase, gonadotroph cells of the anterior pituitary gland secrete LH and FSH in response to pulsatile GnRH stimulation. Circulating LH and FSH promote growth and maturation of ovarian follicles. Developing follicles secrete increasing amounts of estrogen. At first, the estrogen has an inhibitory effect on gonadotropin release. At the midpoint in the menstrual cycle, however, estrogen exerts a brief positive feedback effect on LH and FSH release. This causes follicular rupture and release of an egg into the fallopian tube. During the second half of the cycle, the corpus luteum secretes both estrogen and progesterone. Progesterone induces a change in the endometrium from a proliferative to a secretory type. If fertilization and implantation of a blastocyst does not occur within 14 days after ovulation, the corpus luteum involutes, secretion of estrogen and progesterone declines, menses occurs, and a new cycle begins.

to become atretic, so that usually only one follicle matures. At the same time, increased estrogen levels stimulate the uterine endometrium to proliferate rapidly in preparation for implantation of a fertilized egg.

As the dominant follicle continues to grow, it secretes high, sustained levels of estrogen. Although the mechanism is still not completely understood, the combination of high estrogen levels and the rapid rate of increase of estrogen levels causes a brief positive feedback effect on gonadotroph release of gonadotropins, stimulating rather than inhibiting release of FSH and LH. The resulting midcycle surge of LH and FSH stimulates the dominant follicle to swell and to increase the activity of its proteolytic enzymes, resulting in follicle rupture and ovulation. The ovum is released into the peritoneal cavity and is then taken up by a fallopian tube, where it begins its route toward the uterus.

The cellular remains of the ruptured ovarian follicle involute and become the corpus luteum. Cells of the corpus luteum secrete estrogen and progesterone, not just estrogen. The presence of progesterone in the second half of the menstrual cycle causes the endometrium to switch from a proliferative to a secretory state. The endometrium begins synthesizing proteins necessary for implantation of a fertilized egg. The blood supply to the endometrium also increases to provide increased nutrients if pregnancy ensues.

The corpus luteum has a life span of approximately 14 days. If fertilization and implantation of a viable blastocyst do not occur within 14 days of ovulation, the corpus luteum becomes atretic and ceases its production of progesterone. Without the trophic effects of progesterone, the endometrial lining sheds and menstruation begins. In the absence of estrogen and progesterone, the inhibition of gonadotrophs is removed, and production of FSH and LH rises. This stimulates the development of new ovarian follicles and the beginning of another menstrual cycle.

If fertilization does occur, however, implantation within the uterine lining causes the blastocyst to secrete **human chorionic gonadotropin** (hCG). The presence of hCG stimulates the corpus luteum to remain viable and continue secreting progesterone. Because hCG is one of the first proteins produced by the embryo that is unique to pregnancy, pregnancy tests assay for the presence of hCG. hCG Production decreases after 18 weeks of pregnancy, when the placenta begins to secrete progesterone autonomously. Special considerations attending the use of drugs in pregnancy are discussed in Box 27-1.

## PATHOPHYSIOLOGY

Pathophysiologic processes in the reproductive tract reflect one of three general mechanisms of dysregulation (Table 27-1). The first is disruption of the hypothalamic-pituitary–reproductive axis, which causes a number of underlying disorders that can lead to infertility. The second is inappropriate growth of estrogen-dependent or testosterone-dependent tissue. This can lead to breast cancer or prostate cancer, as well as to benign but clinically important conditions such as endometriosis or endometrial hyperplasia. Finally, decreased estrogen secretion, as in menopause, or decreased androgen secretion is associated with a number of undesirable health consequences.

### BOX 27-1. Application to Therapeutic Decision-Making: Drugs in Pregnancy

(by Vivian Gonzalez Lefebre and Robert H. Rubin)

Pregnancy introduces several special considerations in therapeutic decision-making. These factors include: the health of the woman as well as the delivery of a healthy baby; the altered pharmacokinetics and pharmacodynamics associated with pregnancy; and a lack of information regarding the effects of drugs on the developing fetus.

Most drugs are labeled with disclaimers regarding their use during pregnancy. This paucity of data makes it difficult to estimate the risk-benefit ratio for use of a drug in pregnancy. Physicians depend partly on animal studies and epidemiologic studies (which may be fraught with confounding factors) to establish the teratogenic potential of a drug. **Teratogenesis** refers to the structural or functional dysgenesis of developing organs; each tissue and organ of a fetus has a critical period during which its development may be disrupted by the administration of a teratogenic drug.

The FDA has established a system that classifies drugs on the basis of human and animal data, ranging from class A (safe) to class X (proven teratogenicity) drugs. For example, methyldopa has an excellent safety record in the treatment of hypertension during pregnancy; it is, therefore, considered a class A drug for use in pregnancy. In contrast, ACE inhibitors (another class of antihypertensives) are absolutely contraindicated during the second and third trimesters of pregnancy (class X) because of their association with fetal and neonatal renal dysfunction, including oligohydramnios, neonatal anuria, and renal failure. This classification system is helpful when a drug fits one of the two extremes; classifications in the middle, though, are often confusing and ambiguous. The physician, therefore, relies heavily on clinical judgment to decide whether a drug's potential benefits to the mother outweigh the risk to the fetus. Often, physicians err on the side of not treating.

The following issues should always be addressed when prescribing a drug to a pregnant woman:

- the probability of placental transfer, given the drug's molecular weight, charge, and solubility
- a physiologic explanation for how the drug can affect the fetus, e.g., through effects on organogenesis, organ development, organ function, or a delivery complication
- the risk to both fetus and mother associated with the underlying maternal illness

When assessing the risk-benefit ratio for administering a drug, it should also be recognized that there are drugs that have teratogenic effects in animals when administered in high doses, but that do not present a risk to humans when given in clinical doses. Furthermore, it is important to be aware that the population baseline risk of birth malformations is 3%–5%. When appropriate, drugs that have proved effective for treating a patient's underlying condition should be continued, and experimenting with new drugs should be avoided. Finally, to minimize fetal risk, drugs should be prescribed at the lowest therapeutic dose, taking into account the normal metabolic and physiologic changes that occur during pregnancy (e.g., placental metabolism; increased water retention, renal filtration, heart rate, and plasma volume).

## DISRUPTION OF THE HYPOTHALAMIC-PITUITARY–REPRODUCTIVE AXIS

The hypothalamic-pituitary–reproductive axis is normally tightly regulated via feedback inhibition or stimulation of hormone activity, with the goal of producing a successful menstrual cycle every month. When this axis becomes disrupted, infertility can result. Common causes of infertility caused by disruption of sex hormone production include polycystic ovarian syndrome and prolactinomas.

**Polycystic ovarian syndrome** (PCOS) is a complex syndrome characterized by anovulation and by increased levels of serum androgen. The diagnosis is typically clinical, as in the case of Amy Johnston, and based on the concurrent findings of anovulation and hirsutism (excessive hair growth). Although multiple etiologies are likely to be responsible for

TABLE 27-1 General Mechanisms of Reproductive Tract Disorders for which Pharmacologic Agents are Currently Used

| MECHANISM | EXAMPLES |
|---|---|
| Disruption of the hypothalamic-pituitary–reproductive axis | Polycystic ovarian syndrome<br>Prolactinoma |
| Inappropriate growth of hormone-dependent tissue | Breast cancer<br>Prostatic hyperplasia, prostate cancer<br>Endometriosis, endometrial hyperplasia<br>Leiomyomas (uterine fibroids) |
| Decreased estrogen or androgen secretion | Hypogonadism<br>Menopause |

PCOS, all of the etiologies result in increased androgen secretion and suppression of normal ovulatory cycles. The increased androgen secretion results in masculinization; as seen in Amy's case, male pattern baldness and inappropriate hair growth are common. Many women with PCOS are treated with an anti-androgen, such as spironolactone (see below) to abrogate the masculinizing effects of increased serum testosterone levels.

Three primary hypotheses attempt to explain the development of PCOS. The first, referred to as the **LH hypothesis,** is based on the observation that many women with PCOS have an increased frequency and amplitude of pituitary LH pulses. Increased LH activity could stimulate thecal cells of the ovary to synthesize increased amounts of androstenedione, a potent androgen. In addition, the increased LH levels could alter normal cycling, leading to anovulation. As seen in the introductory case, patients with PCOS menstruate irregularly, and the menstrual periods that they do have tend to be severe. The second hypothesis, referred to as the **insulin theory,** is based on the observation that many women with PCOS are insulin resistant and secrete increased insulin. Insulin decreases the production of sex hormone binding globulin (SHBG). A decrease in plasma SHBG results in a higher concentration of free testosterone and, therefore, greater androgenic effects on peripheral tissues. It has also been observed that insulin can directly synergize with LH to increase androgen production by thecal cells. The third hypothesis is the **ovarian hypothesis.** This explanation posits dysregulation of sex steroid synthesis at the level of the thecal cell. For example, an abnormal increase in the activity of the oxidative enzymes responsible for androgen synthesis could lead to greater thecal cell production of androgens for any given stimulus. It is important to note that these hypotheses are not mutually exclusive, and that PCOS could result from a combination of two or three mechanisms. When the cellular mechanisms underlying this disease are better elucidated, new pharmacologic treatments can potentially be developed that treat the etiology, rather than the effects, of the disease.

**Prolactinomas** are another common cause of infertility among women of reproductive age. An anterior pituitary gland tumor of lactotrophs (prolactinoma) can cause infertility through two parallel pathways. First, increased prolactin levels suppress estrogen synthesis, both by antagonizing the hypothalamic release of GnRH and by decreasing gonadotroph sensitivity to GnRH. This antagonism decreases LH and FSH release and, thereby, decreases end-organ stimulation by the hypothalamic-pituitary–reproductive axis. The second mechanism, common to all pituitary gland tumors, is a crowding-out effect, so that the proliferation of lactotrophs in the anterior pituitary gland inhibits the function of nearby gonadotroph cells.

## INAPPROPRIATE GROWTH OF HORMONE-DEPENDENT TISSUES

Breast and prostate tissue depend on estrogen and testosterone, respectively, for growth. Many (but not all) breast cancers express the estrogen receptor (ER), and the growth of such cancers is often stimulated by endogenous levels of estrogen. When a **breast carcinoma** is found to express the ER, an estrogen receptor antagonist is commonly administered to slow tumor growth. Testosterone-dependent prostate growth requires the local conversion of testosterone to dihydrotestosterone in stromal cells of the prostate; this conversion is mediated by the type II isoform of 5α-reductase. Both enzyme inhibition and receptor antagonist strategies are used to treat conditions in which the growth of prostate tissue is dysregulated, such as **benign prostatic hyperplasia** and metastatic **prostate cancer** (see below).

**Endometriosis** is the benign ectopic growth of endometrial tissue. The fact that endometriosis is usually found in areas surrounding the fallopian tube (ovaries, rectovaginal pouch, and uterine ligaments) has led to the hypothesis that endometriosis could result from retrograde migration of endometrial tissue during menstruation. Other etiologies are possible, however, including metaplastic tissue growth from the peritoneum, or spread of endometrial cells from the uterus to the GI tract via lymphatic ducts. There is also evidence of increased aromatase activity in endometrial tissue from such patients. Because foci of endometriosis respond to estrogen stimulation, endometriosis grows and regresses with the menstrual cycle. This can lead to severe pain, abnormal bleeding, and the formation of adhesions in the peritoneal cavity. Adhesion formation can, in turn, lead to infertility. Because endometriosis is usually estrogen-dependent, treatment with long half-life GnRH agonists often achieves regression of the disease (see below).

## DECREASED ESTROGEN OR ANDROGEN SECRETION

The effects of decreased sex hormone production vary depending on the age of the patient at the onset of symptoms. **Hypogonadism** results if sex hormone production is impaired before adolescence. Patients with hypogonadism do not undergo sexual maturation, but proper hormone replacement can, in many cases, allow the development of secondary sexual characteristics.

**Menopause** is a normal physiologic response to exhaustion of the ovarian follicles. Throughout a woman's lifetime, follicles are arrested in meiosis. Only a small percentage of follicles mature during the menstrual cycle; the rest eventually become atretic. Menstrual cycles cease when all of the follicles are depleted from the ovaries. Follicle depletion leads to a decrease in estrogen (because developing follicles are the main estrogen source in premenopausal women) and an increase in LH and FSH (because estrogen inhibits gonadotropin release). *After menopause, estrone, derived from peripheral aromatization of androstenedione, becomes the primary estrogen present in the blood.* Estrone is a less potent estrogen than estradiol. Because of the relative lack of estrogen after menopause, many women experience hot flashes, vaginal dryness, decreased libido, and dermal atrophy, and often develop osteoporosis. The role of estrogen in the maintenance of bone composition is discussed in further detail in Chapter 29.

Men do not experience a sudden decrease in sex hormones in a manner analogous to the female menopause, but androgen secretion does decline gradually with age. Although controversy currently exists over the role of androgen therapy in normal elderly men, androgen replacement is indicated in cases of adult hypogonadism.

# PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic agents have been developed to target most of the steps in gonadal physiology and pathophysiology. The relevant drug classes include modulators of anterior pituitary gland gonadotroph activity and specific antagonists of peripheral hormone action. In addition, sex hormones are often used as replacement therapy or to modify gonadotropin release (Fig. 27-6).

## INHIBITORS OF GONADAL HORMONES

### Synthesis Inhibitors

#### *GnRH Agonists*

Under physiologic conditions, the hypothalamus releases GnRH in a pulsatile fashion. The frequency of GnRH pulses controls the relative release of LH and FSH by the anterior pituitary gland. In contrast, continuous administration of GnRH suppresses, rather than stimulates, pituitary gonadotroph activity. Taking advantage of this mechanism, it is possible to suppress the hypothalamic-pituitary–gonadal axis either by continuous administration of a GnRH agonist or by administration of a GnRH antagonist. Continuous administration of a GnRH agonist is used to treat such hormone-dependent tumors as prostate cancer and, in some cases, breast cancer. In contrast, short-acting agents can be administered intermittently to simulate physiologic GnRH pulses and, thereby, to promote ovulation. Individual agents are discussed in detail in Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland.

#### *5α-Reductase Inhibitors*

**Finasteride** is a selective inhibitor of type II 5α-reductase, the enzyme that converts testosterone to dihydrotestosterone. The type II reductase is highly expressed in prostate epithelial cells. Recall that dihydrotestosterone binds to the androgen receptor with higher affinity than testosterone. *Blocking the local conversion of testosterone to dihydrotestosterone effectively abrogates the action of testosterone.* Prostate cells are dependent on androgen stimulation for survival, and administration of a reductase inhibitor slows the growth of prostate tissue. Finasteride is currently approved for use in benign prostatic hyperplasia, primarily to improve symptoms of decreased urine flow. The drug is a potential alternative to transurethral resection of the prostate (TURP), which is a common surgical treatment for symptomatic prostatic hyperplasia. One year of therapy with finasteride can result in up to 25% reduction in prostate size. Finasteride is most effective for patients with larger prostates, because the greatest clinical changes are observed in prostates that are already significantly hypertrophied. Troublesome side effects include decreased libido and erectile dysfunction.

#### *Aromatase Inhibitors*

Because estrogens are synthesized from androgen precursors via the action of aromatase, blocking the aromatase enzyme can effectively inhibit estrogen formation. This approach can be used to inhibit the growth of estrogen-dependent tumors, for example, breast cancer. The first agent to be developed in this category was **aminoglutethimide.** Unfortunately, aminoglutethimide has low specificity for aromatase; at therapeutic concentrations, the drug also inhibits side-chain cleavage enzyme, which catalyzes the initial step in adrenal steroid hormone synthesis. Aminoglutethimide use

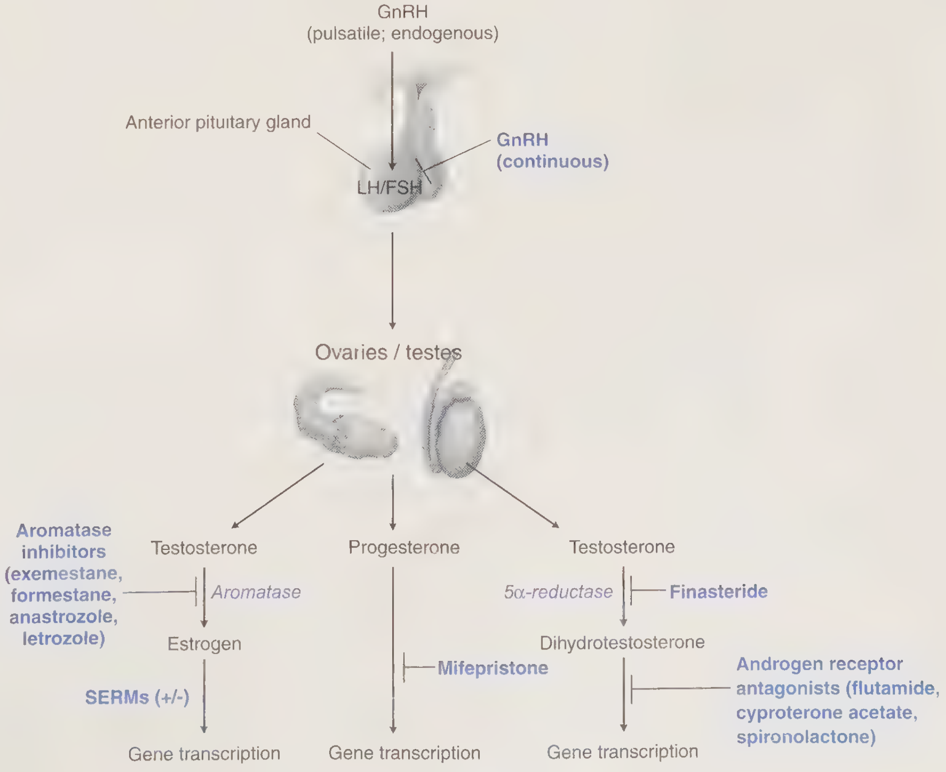


**Figure 27-6. Pharmacologic Modulation of Gonadal Hormone Action.** Pharmacologic modulation of gonadal hormone action can be divided into inhibitors of hormone synthesis and hormone receptor antagonists. Continuous administration of GnRH suppresses LH and FSH release from the anterior pituitary gland, thus preventing gonadal hormone synthesis. GnRH antagonists are also being investigated. Finasteride inhibits the enzyme 5α-reductase, thus preventing conversion of testosterone to the more active dihydrotestosterone. Aromatase inhibitors (exemestane, formestane, anastrozole, letrozole) inhibit production of estrogens from androgens. Finally, there are a number of hormone receptor antagonists that inhibit the action of endogenous estrogens (some SERMs), androgens (flutamide, cyproterone acetate, spironolactone), and progesterone (mifepristone).

is, therefore, accompanied by profound depression of glucocorticoid and aldosterone synthesis. Recently, a number of more selective aromatase inhibitors have been developed. **Anastrozole** and **letrozole** are competitive inhibitors of aromatase, while **exemestane** and **formestane** bind covalently to aromatase (and are, therefore, suicide inhibitors of the enzyme). All of these agents are currently used in the treatment of metastatic breast cancer that is resistant to the estrogen receptor antagonist tamoxifen (see below). A large, recently-completed clinical trial has suggested that treatment with letrozole can significantly reduce the risk of recurrence of breast cancer in patients with estrogen receptor-positive cancer, even after a 5-year course of tamoxifen (see below).

## Receptor Antagonists

### *Selective Estrogen Receptor Modulators*

The term "Selective Estrogen Receptor Modulator" (SERM) is based on the observation that certain so-called anti-estrogen drugs are not pure antagonists, but rather mixed agonists/antagonists (Table 27-2). These pharmacologic agents inhibit estrogenic effects in some tissues, while promoting estrogenic effects in others. The basis for tissue selectivity may include several mechanisms. First, there are two estrogen receptor subtypes, ER$\alpha$ and ER$\beta$, and the expression of these receptor subtypes is tissue-specific. Second, the ability of the estrogen receptor to interact with other transcription cofactors depends on the structure of the ligand that is bound to the receptor. Figure 27-7 provides an example. Assume that the binding of 17$\beta$-estradiol (called "Estrogen" in the figure) to the estrogen receptor causes a conformational change in the receptor, so that two transcriptional cofactors, X and Y, can also bind to the receptor. This complex can then activate three genes: an X-dependent gene, a Y-dependent gene, and a gene that depends on both X and Y. In contrast, the binding of a SERM to the estrogen receptor causes a different conformational change in the receptor, so that the transcription factor X is able to bind, but the transcription factor Y is not. As a result, the SERM–receptor-X complex can activate the X-dependent gene, but not the Y-dependent gene or the (X+Y)-dependent gene.

In addition, assume that transcription factors X and Y are expressed in bone cells, but that breast cells express only transcription factor Y. In the breast, this SERM acts as an antagonist, because: (1) the inability of Y to associate with the SERM-estrogen receptor complex prevents the SERM from activating any estrogen-dependent effects; and (2) binding of the SERM to the estrogen receptor competitively inhibits the binding of endogenous estrogen to the receptor. In bone, however, this SERM acts as a partial agonist because it can activate X-dependent but not Y-dependent genes.

These tissue-specific actions of SERMs have important implications for both the desired effects and the side effects of pharmacologic agents. If it were possible to design a SERM that inhibits estrogen-dependent growth of breast carcinoma without causing estrogen-induced endometrial hyperplasia, then the undesirable side effects of tamoxifen (discussed below) could be reduced. Many investigational SERMs are under development, and it is likely that refined SERM specificity will, in the near future, have important implications for the treatment of osteoporosis, breast cancer, and perhaps even cardiovascular disease. The four SERMs in current clinical use are tamoxifen, toremifene, raloxifene, and clomiphene.

**Tamoxifen** and **toremifene** are SERMs used in the treatment and prevention of breast cancer. The agents have equal efficacy, but tamoxifen is used more frequently because there is greater clinical experience with this agent. Tamoxifen has been employed in the palliative treatment of metastatic breast cancer and, more recently, as adjuvant therapy after lumpectomy. *Tamoxifen is an estrogen receptor antagonist in breast tissue, but a partial agonist in the endometrium.* These pharmacodynamic effects result in inhibition of the estrogen-dependent growth of breast cancer, but also stimulation of endometrial growth. Because of the latter effect, tamoxifen administration is associated with a 4–6-fold increase in the incidence of endometrial cancer. However, tamoxifen is also highly efficacious; the drug increases 5-year survival after surgery for breast carcinomas that test positive for estrogen receptor expression, and it can reduce the incidence of a new primary cancer in the contralateral breast. Because of the side effect of endometrial stimulation, tamoxifen is typically administered for no more than 5 years, in order to minimize the risk of iatrogenic endometrial cancer.

**Raloxifene** is a newer SERM that possesses *estrogen receptor agonist activity in bone, but antagonist activity in breast and endometrial tissue.* Consistent with this profile of tissue specificities, raloxifene does not seem to increase the incidence of endometrial cancer. The agonist activity of raloxifene in bone decreases bone resorption and, thus, delays or prevents the progression of osteoporosis in postmenopausal women (discussed in more detail in Chapter 29).

**TABLE 27-2** Tissue-Specific Agonist and Antagonist Activity of Selective Estrogen Receptor Modulators (SERMs)

| | BREAST | ENDOMETRIUM | BONE |
|---|---|---|---|
| Estrogen | +++ | +++ | +++ |
| Tamoxifen/Toremifene | – | + | + |
| Raloxifene | – | – | ++ |

Estrogen, the physiologic hormone, has stimulatory effects in breast, endometrium, and bone. Tamoxifen and toremifene are antagonists in breast tissue and are, therefore, used in the treatment of estrogen receptor-positive breast cancer. Raloxifene, the newest SERM, is an agonist in bone but an antagonist in the breast and endometrium. For this reason, raloxifene is approved for the prevention and treatment of osteoporosis in postmenopausal women. Clomiphene (not shown in the table) is a SERM that acts as an estrogen receptor antagonist in the hypothalamus and anterior pituitary gland; it is used clinically to induce ovulation.

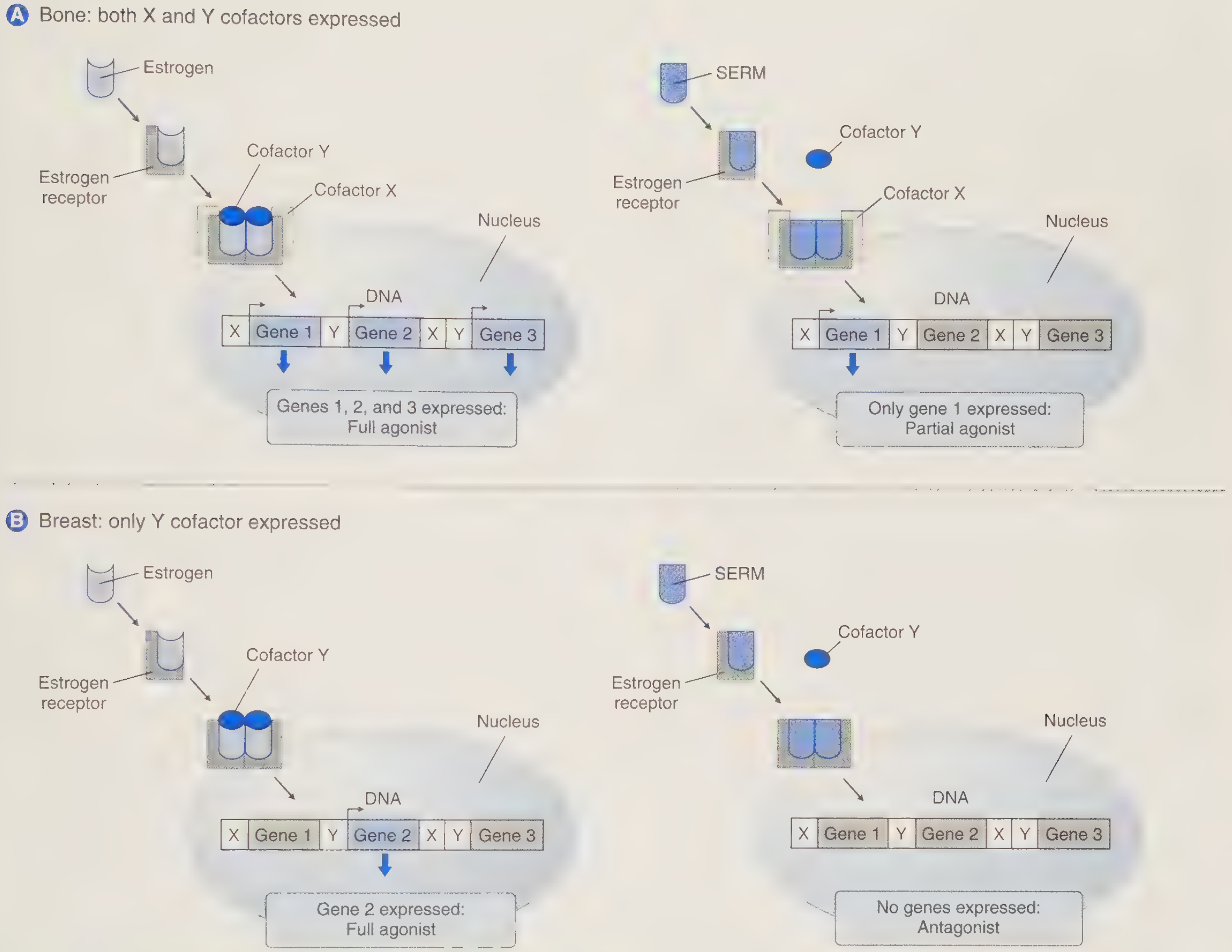

A Bone: both X and Y cofactors expressed
Estrogen
Estrogen receptor
Cofactor Y
Cofactor X
Nucleus
DNA
X Gene 1 Y Gene 2 X Y Gene 3
Genes 1, 2, and 3 expressed: Full agonist
SERM
Cofactor Y
Estrogen receptor
Cofactor X
Nucleus
DNA
X Gene 1 Y Gene 2 X Y Gene 3
Only gene 1 expressed: Partial agonist
B Breast: only Y cofactor expressed
Estrogen
Estrogen receptor
Cofactor Y
Nucleus
DNA
X Gene 1 Y Gene 2 X Y Gene 3
Gene 2 expressed: Full agonist
SERM
Cofactor Y
Estrogen receptor
Nucleus
DNA
X Gene 1 Y Gene 2 X Y Gene 3
No genes expressed: Antagonist

**Figure 27-7. A Model for the Tissue Specificity of Action of SERMs.** Selective estrogen receptor modulators (SERMs) exhibit tissue-specific estrogen receptor antagonist or partial agonist activity. This tissue specificity of action seems to be explained by the following observations: (1) transcriptional coactivators and/or corepressors are expressed in a tissue-specific manner; (2) a SERM-estrogen receptor (ER) complex can associate with some co-activators or co-repressors, but not others; and (3) genes can be activated or inhibited by different combinations of SERM-ER and co-activators or co-repressors. In the example shown, assume that bone cells express co-activators (co-factors) X and Y, whereas breast cells express only coactivator Y. The estrogen–ER complex can associate with X and Y, whereas the SERM–ER complex can associate with only X. **A,** In bone cells, estrogen binding to ER and coactivators X and Y induces expression of genes 1, 2, and 3. The SERM–ER complex cannot bind coactivator Y; however, and the SERM–ER-cofactor X complex induces expression of only gene 1. In bone, then, estrogen is a full agonist, whereas the SERM is a partial agonist. **B,** In breast cells, estrogen binding to ER and coactivator Y induces expression of gene 2, but the SERM is unable to promote expression of any gene. In breast, then, the SERM acts as an antagonist. For simplicity, this model shows only co-activators, although corepressors are also involved in SERM action.

Raloxifene is currently approved only for the treatment and prevention of osteoporosis. However, the drug's anti-estrogen effects in breast tissue, and its inability to stimulate endometrial growth, may make raloxifene an attractive drug for the prevention of breast cancer. Prospective clinical trials are currently underway to assess the efficacy of raloxifene for prevention of breast cancer and heart disease.

**Clomiphene** is a SERM used to induce ovulation. *The drug acts as an estrogen receptor antagonist in the hypothalamus and anterior pituitary gland, and as a partial agonist in the ovaries.* The antagonist activity of clomiphene in the hypothalamus and anterior pituitary gland results in relief of the negative feedback inhibition imposed by endogenous estrogen and, therefore, in the increased release of GnRH and gonadotropins, respectively. The increased levels of FSH and LH simulate the LH surge that characterizes the middle of the menstrual cycle and, thus, promote ovulation. Clomiphene's main side effect is that it can increase ovary size, because of both the increased follicular development induced by LH and FSH stimulation, and the estrogen agonist effects of clomiphene itself on the ovaries. Unlike the administration of exogenous FSH (see Chapter 24), however, clomiphene use is not associated with the ovarian hyperstimulation syndrome; the reasons for this are unknown.

### Androgen Receptor Antagonists

Androgen receptor antagonists competitively inhibit the binding of endogenous androgens to the androgen receptor. By this mechanism, receptor antagonists block the action of testosterone and dihydrotestosterone on their target tissues. The androgen receptor antagonists include **cyproterone acetate** and **flutamide.** Cyproterone acetate is often used in the treatment of hirsutism in women, and is also under study as a form of male contraception (see below). Flutamide is approved only for the treatment of metastatic prostate cancer, but the drug is also used therapeutically in the treatment of benign prostatic hyperplasia. **Spironolactone,** originally approved as an aldosterone receptor antagonist (see Chapter 19, Pharmacology of Volume Regulation), also has significant antagonist activity at the androgen receptor. Like cyproterone acetate, therefore, spironolactone can be used as a competitive inhibitor of testosterone action. Ms. Johnston was treated with spironolactone to antagonize the excessive androgen stimulation of her hair follicles and, thus, to ameliorate her hirsutism.

### *Progesterone Receptor Antagonists*

**Mifepristone** (also referred to as **RU-486**) is a progesterone receptor antagonist used to induce first-trimester abortion. As noted above, progesterone is crucial for maintenance of the endometrium during pregnancy; the hormone stabilizes the uterine lining and promotes vessel growth and secretory activities of the decidua. Mifepristone inhibits progesterone action by binding competitively to the progesterone receptor. Blockade of progesterone action results in decay and death of the decidua, and lack of nourishment from the decidua causes the blastocyst to die and detach from the uterus. Because the blastocyst is no longer secreting hCG, the corpus luteum involutes, and involution of the corpus luteum causes progesterone synthesis and secretion to decrease. Overall, then, mifepristone treatment initiates a positive feedback loop in which blockade of progesterone action leads to a marked decrease in progesterone synthesis. This sequence of events may explain the high efficacy of mifepristone.

Mifepristone is commonly administered in conjunction with **misoprostol,** a prostaglandin analogue (see Chapter 40, Pharmacology of Eicosanoids). Misoprostol stimulates uterine contractions, and the combined effects of progesterone antagonism and uterine contractions are more than 95% effective in terminating first-trimester pregnancy.

Because mifepristone is administered as a single dose, side effects related to progesterone antagonism are rare. Instead, the main potential for complication lies in the subsequent abortion, which can result in excessive vaginal bleeding. In addition, co-administration of misoprostol can cause nausea and vomiting. If medical abortion with mifepristone and misoprostol is unsuccessful, then the pregnancy should be terminated by other means, because misoprostol can cause congenital abnormalities in fetuses carried to term.

## HORMONES AND HORMONE ANALOGUES: CONTRACEPTION

The development of safe, efficacious contraceptives for women has revolutionized sexual practices. The two classes of widely used oral contraceptives are **estrogen/progesterone combinations** and **progestin-only contraception.** The development of male contraception is an active area of research; current approaches to this therapy are discussed briefly at the end of the section.

### Combination Oral Contraception

Combination oral contraception suppresses follicular development and, thereby, inhibits ovulation and prevents fertilization. Mechanistically, the administration of exogenous estrogen inhibits gonadotroph release of FSH and LH. Suppression of gonadotropin release prevents the midcycle LH surge, so ovulation never occurs. Co-administration of estrogen and progestin may also inhibit pregnancy by a number of secondary mechanisms, including alteration of tubal peristalsis and changes in cervical mucus secretions. The latter actions could together prohibit the proper transport of both egg and sperm, even if ovulation were to occur. *In combination, these mechanisms explain the >99% efficacy of combination oral contraception.*

The estrogen used in oral contraceptive pills (OCPs) is either **ethinyl estradiol** or **mestranol.** Use of "unopposed" estrogens promotes endometrial growth, and early studies of estrogen-only contraception determined that these agents increase the risk of endometrial cancer. Because of this finding, estrogen is always co-administered with a progestin to limit the extent of endometrial growth. Ideally, the progestin would possess activity only at progesterone receptors, but all currently available progestins also have some androgenic cross reactivity. **Norgestrel** and **levonorgestrel** have high androgenic activity, while **norethindrone** and **norethindrone acetate** have lower androgenic activity. The so-called third-generation progestins **ethynodiol, norgestimate,** and **desogestrel** have even lower androgen receptor cross reactivity. The latter agents are not commonly used, however, because concern exists that the third-generation progestins may be associated with an increased risk of deep vein thrombosis. For this reason, norethindrone and norgestrel are currently the preferred progestins for combination oral contraception.

Current regimens of combination oral contraception consist of 21 days of drugs followed by 7 days of a placebo pill. The seven-day placebo period removes exogenous hormone stimulation, simulating the physiologic involution of the corpus luteum that occurs at the end of a normal menstrual cycle. The lack of estrogen and progestin causes the endometrium to slough, resulting in menstruation. Because the administration of progestin throughout the cycle inhibits the proliferative growth of the endometrium, most women experience lighter menstrual periods when taking OCPs, and a woman's menstrual cycle often becomes more regular. Recently, the FDA approved a longer cycle formulation of ethinyl estradiol and levonorgestrel, in which the drug combination is administered for 84 days followed by 7 days of placebo. The formulation appears to have equal contraceptive efficacy, and reduces to four the total number of menstrual cycles per year.

Combination contraceptive formulations consist of monophasic, biphasic, or triphasic hormone schedules. The standard formulation, used by the majority of women, is a constant (monophasic) dose of estrogen and progestin for 21 days. Biphasic preparations maintain a constant estrogen dose throughout the cycle, while the progestin is initially low but then increases during the second half of the cycle. Triphasic formulations incorporate both increased progestin in the latter half of the cycle and a midcycle increase in the estrogen dose, to prevent breakthrough bleeding. *The main advantage of biphasic or triphasic administration is that the total amount of progestin administered over each month is reduced.* Indeed, the general trend in recent years has been to decrease the quantities of administered estrogen and progestin to the smallest amount necessary for inhibition of ovulation. However, there are no clearly established differences in either the side effects or the clinical efficacy of monophasic compared to biphasic or triphasic therapy.

A number of studies have been performed to assess the side effects of long-term contraceptive use. These studies have shown that the incidence of **deep vein thrombosis** and the incidence of **pulmonary embolism** are increased with OCPs, but that these complications occur so infrequently that the absolute number of adverse events is low. Studies have failed to demonstrate any increase in breast cancer. As noted above, unopposed estrogen administration does increase the incidence of endometrial cancer. Use of oral contraceptives is associated with an increase in **gallbladder disease,** because estrogens increase the biliary concentration of cholesterol relative to that of bile salts, and the resulting decrease in cholesterol solubility promotes the formation of gallstones. *Oral contraceptives should not be administered to women over 35 who smoke, because the administration of contraceptives to this population is associated with an increase in thrombotic cardiovascular events.*

Recent studies have focused on the benefits rather than the side effects of oral contraception. Modern combination oral contraceptives *reduce* the incidence of endometrial cancer, probably because constant administration of a progestin inhibits endometrial growth. In addition, exogenous administration of an estrogen/progestin combination reduces the incidence of ovarian cancer, probably by lowering circulating levels of gonadotropins. *Overall, the consensus is that oral contraceptives have more beneficial than harmful medical effects.*

### Morning-After Contraception

Combination oral contraceptives can be used as emergency postcoital contraception after unprotected sex or failure of a contraceptive device. "Morning-after" contraception consists of two doses of estrogen and progestin that are each approximately twice the dose used in a normal combination oral contraceptive pill. The exact mechanism of action of postcoital estrogen and progestin is unknown, but it is thought that the hormones could interfere with ovulation and/or transport of a fertilized egg to the uterus. Regardless of the mechanism, high-dose estrogen and progestin has a reported efficacy of 75–80% when taken within 72 hours of intercourse. Nausea is a common side effect of the large estrogen doses, and these drugs are therefore generally co-prescribed with an antinausea agent.

### Progestin-Only Contraception

A newer approach to female contraception involves the continuous administration of low-dose progestins. The two progestin-only contraceptives available in the United States are **norgestrel** and **norethindrone.** These drugs can be administered as tablets commonly referred to as the "minipill." Norgestrel is also available as a subdermal implant that slowly releases progestin into the bloodstream.

Progestin-only contraception prevents ovulation 70–80% of the time, probably because progestins alter the frequency of GnRH pulsing and decrease pituitary gland responsiveness to GnRH. Despite the relatively high frequency of ovulation, this form of contraception is 96–98% effective, suggesting that secondary mechanisms, such as alterations of cervical mucus and tubal peristalsis, are probably also at work. Because progesterone inhibits endometrial proliferation and promotes endometrial secretion, it may also be the case that an egg is unable to implant in an endometrium that is continually exposed to progestin. Patients taking these drugs do not typically menstruate, but breakthrough spotting and irregular, light menstrual periods commonly occur during the first year of administration.

### Male Contraception

The development of an efficacious male contraceptive has been studied for years, but has not yet met clinical success. The goal of male contraception would be to suppress endogenous production of sperm reversibly, generating a state of azoospermia (absence of sperm in the ejaculate) without suppressing libido or erectile function. Reliable inhibition of spermatogenesis is a difficult task, because even a 99% reduction in spermatogenesis would result in a sufficient number of viable sperm for fertilization.

Initial studies of male contraception centered on the administration of **testosterone enanthate** (see below). As an end-product of the hypothalamic-pituitary–gonadal axis, testosterone significantly suppresses gonadotropin release. The reduced circulating levels of LH and FSH are unable to stimulate Sertoli cell function, and decreased spermatogenesis results. The main difficulties with this approach have been the large population variability in the degree of spermatogenesis inhibition (on average, only 60% of men become azoospermic), and the significant side effects of acne, weight gain, and polycythemia.

Because many of the side effects of testosterone could be dose-related, combination therapy has been attempted with co-administration of an androgen and a progestin. Progestins have independent suppressive effects on pituitary gland gonadotroph activity and, thus, could act synergistically with testosterone to suppress gonadotropin release. As with the androgen-only studies, however, these studies have not yet identified a combination that reliably produces reversible azoospermia.

## HORMONES AND HORMONE ANALOGUES: REPLACEMENT

Estrogens and androgens are both used as replacement therapies in cases of hormone deficiency.

### Estrogens

The realization that estrogen loss at menopause has many deleterious effects has led to the development of perimenopausal and postmenopausal hormone replacement therapy (for additional detail, see Chapter 29). The principal indication for such therapy is to suppress hot flashes. Used for this indication, estrogen replacement is highly effective. Recently, the FDA approved a vaginal ring that elutes estradiol at a controlled dose rate (see Chapter 50, Drug Delivery Mechanisms). This local administration of estrogen is an effective therapy for postmenopausal vaginal dryness and atrophy. Estrogen replacement is also effective in reducing the development of osteoporosis in postmenopausal women (see Chapter 29) and can be used to improve vaginal dryness and cutaneous tissue quality. Until recently, hormone replacement therapy was also thought to have beneficial effects on the cardiovascular system in postmenopausal women. However, a large prospective study, the Women's Health Initiative (WHI), has recently demonstrated that the risks of an estrogen/progestin combination exceed the benefits. Specifically, the significantly increased risks of breast cancer, cardiovascular events, and dementia outweigh the beneficial effects of the therapy on the skeleton and a therapy-associated decrease in colon cancer. Therefore, current recommendations are not to prescribe long-term estrogen replacement for the prevention of osteoporosis or cardiovascular disease. It is not yet known whether estrogen alone, in the absence of a progestin, carries the same risk profile as the estrogen/progestin combination.

## Androgens

Androgen replacement is an effective therapy for hypogonadism. Oral testosterone is ineffective because of its high first-pass metabolism by the liver. Two esters of testosterone, **testosterone enenthate** and **testosterone cypionate,** can be administered intramuscularly. A preparation of either of these agents, injected every 2–4 weeks, increases plasma testosterone to physiologic concentrations in hypogonadal men. Recently, transdermal **testosterone patches** have been developed; this drug delivery system has the advantages that plasma testosterone levels remain relatively constant and first-pass hepatic metabolism is bypassed. Testosterone is also available in a topical gel formulation; using this preparation on a once-a-day application schedule, plasma testosterone levels gradually increase until they reach physiologic replacement levels after 1 month of application. Testosterone can also be administered as a tablet that adheres to the buccal mucosa, resulting in rapid systemic absorption of the drug.

Some athletes abuse androgens by self-administration at supratherapeutic levels. Almost every type of androgen has been abused in an attempt to enhance athletic performance, including the adrenal hormone precursors androstenedione and dehydroepiandrosterone. Pharmacologic doses of androgens suppress the hypothalamic-pituitary–gonadal axis, resulting in suppression of testicular function, decreased sperm production, and impaired fertility. Because many androgens can be converted to estrogens by aromatase, pharmacologic doses of androgens can also cause an increase in plasma estrogen, resulting in gynecomastia. In addition, high plasma levels of androgens are associated with erythrocytosis, severe acne, and derangements in lipid metabolism (increased LDL and decreased HDL).

## *Conclusion and Future Directions*

The male and female hormones of reproduction share significant mechanistic overlap with one another. Androgens, estrogens, and progestins are all steroid hormones that exert their physiologic action by binding to intracellular receptors, translocating to the nucleus and altering gene transcription. Recent evidence suggests that estrogens may also act on cell surface receptors to mediate nongenomic effects. Derangements in the physiologic effects of reproductive hormones can involve disruption of the hypothalamic-pituitary–reproductive axis, inappropriate growth of hormone-dependent tissue, or decreased activity of gonadal hormones at target tissues. Currently available pharmacologic agents can modify the endocrine axis (e.g. GnRH), inhibit synthesis of active hormones (e.g. 5α-reductase inhibitors, aromatase inhibitors), or inhibit end-organ effects at the receptor level (e.g. SERMs, anti-androgens, mifepristone). Oral contraceptives, such as estrogen/progestin combinations and progestin-only contraception, disrupt the exquisite chronicity of the menstrual cycle and, thus, suppress ovulation. The development of an effective male contraceptive has met a number of obstacles, but should represent a major pharmacologic advance in the future. Exciting progress is also being made in the design of new SERMs that possess a variety of tissue-specific activities; such research may result in new agents effective for both prevention of breast cancer and treatment of postmenopausal osteoporosis.

## *Suggested Readings*

Cadepond F, Ulmann A, Baulieu EE. RU486 (Mifepristone): Mechanisms of action and clinical uses. Ann Rev Med 1997;48:129–156. (*Discussion of mifepristone indications, actions, and side effects*)

Gustafsson JA. What pharmacologists can learn from recent advances in estrogen signalling. Trends Pharmacol Sci 2003; 24:479–485. (*Current review of estrogen receptor subtypes*)

Handelsman DJ. Hormonal male contraception. Int J Androl 2000; 23:S8–S12. (*Review of different approaches to male contraception*)

McCann MF, Potter LS. Progestin-only oral contraception: A comprehensive review. Contracep 1994;50:S1–S198. (*Comprehensive treatment of progestin-only contraception*)

Osborne C. Tamoxifen in the treatment of breast cancer. N Engl J Med 1998;339:1609–1618. (*Discusses approaches to hormonal treatment of breast cancer*)

Petitti DB. Clinical practice. Combination estrogen-progestin oral contraceptives. N Engl J Med 2003;349:1443–1450. (*Current review of combination oral contraception*)

Weisberg E. Prescribing oral contraceptives. Drugs 1995;49:224–231. (*Overview of the relative advantages and disadvantages of different formulations*)

Weryha G, Pascal-Vigneron V, Klein M, Leclere J. Selective estrogen receptor modulators. Curr Opin Rheum 1999;11:301–306. (*Presents theories underlying tissue-specific estrogen receptor antagonism*)

**Drug Summary Table** **Pharmacology of Reproduction**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **GONADOTROPIN-RELEASING HORMONE (GnRH) AGONISTS**<br>*Mechanism—See Chapter 24 (p. 414)* | | | | |
| **Gonadorelin** | See Chapter 24 | | | |
| **Goserelin**<br>**Histrelin**<br>**Leuprolide**<br>**Nafarelin** | See Chapter 24 | | | |
| **INHIBITORS OF PERIPHERAL TESTOSTERONE CONVERSION TO DHT**<br>*Mechanism—Inhibit 5α-reductase* | | | | |
| **Finasteride** | Benign prostatic hypertrophy | Decreased libido | | Also used to treat male pattern hair loss |
| **INHIBITORS OF AROMATASE**<br>*Mechanism—Inhibit formation of estrogens from androgens* | | | | |
| **Aminoglutethimide** | Metastatic breast cancer | Glucocorticoid deficiency | | Also inhibits initial step in adrenal steroid synthesis |
| **Anastrozole**<br>**Letrozole** | Metastatic breast cancer | Hot flashes, rash | | Selective aromatase inhibitors; used in tamoxifen-resistance |
| **Exemestane**<br>**Formestane** | Metastatic breast cancer | Hot flashes, rash | | Suicide inhibitors of aromatase |
| **SELECTIVE ESTROGEN RECEPTOR MODULATORS**<br>*Mechanism—Estrogen receptor partial agonists; estrogen receptor antagonists (tissue selective)* | | | | |
| **Tamoxifen**<br>**Toremifene** | Breast cancer | Hot flashes, ↓ libido<br>Hypercalcemia, tumor flare | ↑ Risk endometrial cancer | Estrogen antagonists in breast tissue, partial agonists in endometrium and bone |
| **Raloxifene** | Osteoporosis prevention and treatment | Hot flashes, DVT | Pregnancy | Estrogen partial agonist in bone, antagonist in breast and endometrium |
| **Clomiphene** | Induce ovulation | ↑ Ovary size, hot flashes | | Estrogen antagonist in hypothalamus, partial agonist in ovaries |
| **ANDROGEN RECEPTOR ANTAGONISTS**<br>*Mechanism—Inhibit dihydrotestosterone binding to receptor* | | | | |
| **Flutamide**<br>**Cyproterone** | Prostate cancer | Rare liver failure<br>Gynecomastia | | |
| **Spironolactone** | Polycystic ovarian syndrome | Gynecomastia | | |

(Continued)

## Drug Summary Table Pharmacology of Reproduction *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **PROGESTERONE RECEPTOR ANTAGONISTS**<br>*Mechanism—Inhibit progesterone binding to receptor* | | | | |
| **Mifepristone** | Medical abortion | Bleeding | Pregnancy >49 days | Must be able to verify age of fetus<br>Coadministered with misoprostol (causes uterine contractions, nausea)<br>Antagonist at glucocorticoid receptor as well as progesterone receptor |
| **MIXED ESTROGEN/PROGESTIN ORAL CONTRACEPTIVES**<br>*Mechanism—Suppress follicular development; inhibit midcycle surge of LH and FSH; inhibit ovulation* | | | | |
| *Estrogens:*<br>**Ethinyl estradiol**<br>**Mestranol**<br>*Progestins:*<br>**Norgestrel, levonorgestrel**<br>**Norethindrone**<br>**Ethynodiol, norgestimate**<br>**Desogestrel** | Prevention of pregnancy<br>Postcoital contraception<br>Slightly ↑ risk stroke<br>↑ Triglyceride levels<br>↑ Risk DVT | Breakthrough bleeding<br>↑ Blood pressure | *Contraindications:*<br>Previous DVT or stroke<br>History of estrogen-dependent tumor<br>Liver disease<br>Pregnancy<br>Hypertriglyceridemia<br>Women > 35 y/o who smoke<br>*Drug Interactions:*<br>Rifampin, phenytoin, and phenobarbital all ↑ metabolism of OCPs | Formulations exist as monophasic, biphasic, triphasic dosage forms<br>Monophasic: constant estrogen and progestin<br>Biphasic: higher progestin in second half of cycle<br>Triphasic: higher progestin in second half of cycle + midcycle ↑ estrogen<br>No clinical differences in efficacy or side effects among monophasic, biphasic or triphasic |
| **PROGESTIN-ONLY CONTRACEPTIVES**<br>*Mechanism—Altered GnRH release leads to ↓ ovulation* | | | | |
| **Norgestrel**<br>**Norethindrone** | Contraception | Breakthrough spotting | | Norgestrel also available as subdermal implant<br>Less effective than estrogen/progestin combination |
| **PROGESTINS USED IN BREAST CANCER**<br>*Mechanism—Unknown* | | | | |
| **Megestrol acetate**<br>**Medroxyprogesterone acetate** | Advanced breast cancer | ↑ Risk DVT<br>Hot flashes | | |

# 28

# Pharmacology of the Endocrine Pancreas

Aimee D. Shu and Martin G. Myers, Jr.

**Introduction**
**Case**
**Biochemistry and Physiology**
- Pancreatic Anatomy
- Energy Homeostasis
  - *Fed State*
  - *Fasting State*
- Insulin
  - *Biochemistry*
  - *Secretion*
  - *Action at Target Tissues*
- Glucagon
- Somatostatin

**Pathophysiology**
- Diabetes Mellitus
  - *Type I Diabetes*
  - *Type II Diabetes*
  - *Morbidity and Mortality*
- Hyperinsulinemia

**Pharmacologic Classes and Agents**
- Therapy for Diabetes
  - *Strategies of Therapy*
  - *Inhibitors of Intestinal Glucose Absorption: α-Glucosidase Inhibitors*
  - *Insulin Replacement: Exogenous Insulin*
  - *Insulin Secretagogues: Sufonylureas and Meglitinides*
  - *Insulin Sensitizers: Thiazolidinediones and Biguanides*
  - *Combination Therapy*
- Therapy for Hyperinsulinemia
- Glucagon as Therapeutic Agent

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

This chapter presents the physiology and pharmacology of the pancreatic hormones insulin, glucagon, and somatostatin. Because diabetes mellitus—caused by the absence or functional insufficiency of insulin—is clinically the most common disease of these endocrine axes, the majority of the chapter is devoted to the physiology and pharmacology of insulin. Medical students may be interested to note that Charles Best, a fourth-year medical student in Canada, had a significant role in the identification of insulin. Along with his mentor, surgeon Frederick Banting, Best isolated a pancreatic extract from dogs that could reduce blood glucose in diabetic dogs and humans. Although the 1923 Nobel Prize in Medicine or Physiology was jointly awarded to surgeon Frederick Banting and physiologist J. J. R. MacLeod, Banting shared his award with Best.

## Case

At her annual check-up, 55-year-old Mrs. Sweetie complains of fatigue and frequent urination (polyuria), even at night. She also reports drinking large volumes of fluids (polydipsia) to quench her thirst. Although these symptoms have been "going on for a while" and are getting worse, Mrs. Sweetie has difficulty pinpointing their exact onset. She denies other urinary symptoms such as pain on urination, blood in her urine, dribbling, and incontinence. Her past medical history is remarkable for hyperlipidemia of 10 years duration. Both of her parents died of coronary heart disease in their early 60s.

On physical examination, Mrs. Sweetie is moderately obese but otherwise appears normal. Glucose is detected in her urine, but proteins and ketones are not. Mrs. Sweetie's blood tests are significant for elevated glucose (240 mg/dL), elevated total cholesterol (340 mg/dL), and elevated HbA1c (a measure of glucose covalently bound to hemoglobin) (9.2%). The physician explains to Mrs. Sweetie that she might have diabetes mellitus. In this disease, the body fails to respond normally to insulin (insulin resistance) and cannot produce a sufficient amount of insulin to overcome this resistance.

The physician discusses with Mrs. Sweetie the importance of decreasing her caloric intake and increasing her exercise to improve her metabolic state. The physician also prescribes metformin (a biguanide) for Mrs. Sweetie's diabetes.

## QUESTIONS

1. What are the cellular and molecular actions of insulin?
2. What is the etiology of diabetes mellitus, and how is Type I diabetes mellitus different from Type II diabetes mellitus?
3. What do the blood glucose and HbA1c levels report about Mrs. Sweetie's diabetes? Are there circumstances under which one parameter could be elevated and the other could be normal?
4. In addition to alleviating her polyuria and polydipsia, why is it important to control Mrs. Sweetie's diabetes (i.e., what acute and chronic complications could arise)?
5. What are the mechanisms of action of the various pharmacologic agents used to treat diabetes: α-glucosidase inhibitors, sulfonylureas, meglitinides, thiazolidinediones, and biguanides? Which is most appropriate for Mrs. Sweetie?

## BIOCHEMISTRY AND PHYSIOLOGY

### PANCREATIC ANATOMY

The pancreas is a glandular organ that contains both exocrine and endocrine tissue. The exocrine portion—which constitutes 99% of the pancreatic mass—secretes bicarbonate and digestive enzymes into the gastrointestinal (GI) tract. Scattered within the exocrine tissue are nearly one million small islands of endocrine tissue that secrete hormones directly into the blood. These tiny endocrine glands, collectively called **Islets of Langerhans,** include diverse cell types that secrete different hormones. **α cells** release **glucagon; β cells** release **insulin; δ cells** release **somatostatin** and gastrin; and PP cells release pancreatic polypeptide.

### ENERGY HOMEOSTASIS

The storage of nutrients for later release into the circulation allows life to continue in the absence of continuous feeding. Insulin and glucagon are the primary hormones involved in controlling the uptake, utilization, storage, and release of cellular nutrients. Insulin promotes the uptake and storage of glucose and other small, energy-containing molecules. The **"counter-regulatory" hormones**—glucagon, catecholamines (i.e., norepinephrine and epinephrine from the sympathetic nervous system and adrenal medulla), glucocorticoids (i.e., cortisol from the adrenal cortex), and growth hormone (from the pituitary gland)—oppose the action of insulin and promote the release of nutrients (see Table 28-1). Blood glucose is easily measured and provides an accurate guide to the bal-

TABLE 28-1 Effects of Selected Hormones on Energy Homeostasis

| HORMONE | SOURCE | TARGET TISSUES | ACTION |
|---|---|---|---|
| Glucagon | α Cell (pancreas) | Liver (adipose, skeletal muscle) | Promotes glycogenolysis and gluconeogenesis in liver, lipolysis in adipose tissue |
| Insulin | β Cell (pancreas) | Liver (adipose, skeletal muscle) | Promotes uptake of glucose, amino acids, and fatty acids from blood into cells for storage as glycogen, protein, and triglyceride |
| Somatostatin | δ Cell (pancreas)<br>GI tract<br>Hypothalamus | Other islet cells, GI tract, brain and pituitary gland | Decreases release of insulin and glucagon<br>Decreases GI tract motility and hormone release<br>Decreases growth hormone secretion |
| Epinephrine | Adrenal medulla | Many | Promotes glycogenolysis in liver<br>Lipolytic via activation of hormone-sensitive lipase |
| Cortisol | Adrenal cortex | Many | Antagonizes insulin action at target tissues<br>Promotes gluconeogenesis in liver, protein breakdown in muscle |
| Leptin | Adipocytes | CNS (basomedial hypothalamus) | Signals adequacy of body energy stores, decreases food intake, permits energy-intensive neuroendocrine functions |

Physiologically, insulin and glucagon are the two most important hormones controlling glucose homeostasis. Insulin promotes energy storage in target tissues. Glucagon, epinephrine, cortisol, and growth hormone—the "counter-regulatory" hormones—act to raise blood glucose and, thereby, to counteract the effects of insulin. By acting as a "fat sensor," leptin signals total body energy storage and regulates long-term energy balance.

ance of insulin and the counter-regulatory hormones. This balance normally keeps glucose levels within a narrow range (70–120 mg/dL), regardless of recent food intake. Hypoglycemia is dangerous because body organs—particularly the brain—depend on a constant supply of glucose for proper functioning. Conversely, chronic hyperglycemia is toxic to many cells and tissues.

The recently identified hormone **leptin** regulates long-term energy balance and the neuroendocrine response to energy storage. Leptin is secreted from fat cells, and its concentration in the plasma is proportional to total fat mass. Thus, leptin signals to the central nervous system the amount of energy—in the form of adipose tissue—that is stored in the body. Leptin promotes anorexia (decreased eating) and permits the endocrine system to perform energy-expensive functions such as growth, reproduction, and maintenance of a high metabolic rate. Alternatively, the lack of leptin in states of starvation results in increased appetite and impairment of energy-expensive functions.

## Fed State

After a meal, complex carbohydrates are broken down to monosaccharides (e.g., glucose, galactose, and fructose) in the lumen of the GI tract and transported into GI epithelial cells by a combination of passive and active apical membrane transporters. Sugars are then transported by basal membrane transporters from the epithelial cell cytosol to intercellular spaces, from which the sugars continue on into capillaries. When glucose in the bloodstream is taken up by pancreatic β cells, the cells release insulin into capillaries that eventually drain into the portal vein. The liver, therefore, receives the highest concentrations of insulin concurrently with the nutrients that have been absorbed from the digestive tract. *The liver and the other energy-storing tissues, such as skeletal muscle and adipose tissue, are the primary tissue targets for insulin* (Fig. 28-1). Local actions of insulin in the Islets of Langerhans also suppress secretion of glucagon from pancreatic α cells.

## Fasting State

As the plasma glucose concentration decreases, pancreatic α cells release increasing amounts of glucagon, and pancreatic β cells secrete decreasing amounts of insulin. In contrast to insulin, which promotes the cellular uptake of glucose in the fed state, glucagon works to mobilize glucose from the liver by stimulating gluconeogenesis and glycogenolysis. As fasting continues, catecholamine and glucocorticoid levels also increase, promoting the release of fatty acids from adipose tissue and the breakdown of protein to amino acids in muscle.

# INSULIN

## Biochemistry

Insulin is a 51-amino acid protein composed of two peptide chains that are linked by disulfide bridges. Its name comes from the Latin *insula* (meaning "island," after the Islets of Langerhans). The human pancreas contains approximately 8 mg of insulin, of which 0.5–1.0 mg is secreted (and replenished by ongoing synthesis) daily. Insulin is initially synthesized in pancreatic β cells as preproinsulin, which is cleaved in turn to proinsulin and then to insulin and free connecting (C) peptide (Fig. 28-2).

## Secretion

Resting pancreatic β cells are poised to secrete insulin, which is preformed and stored in secretory vesicles just beneath the plasma membrane. The low basal rate of insulin secretion is increased dramatically upon exposure of the cells to glucose. Glucose metabolism increases the intracellular **ATP/ADP ratio,** which stimulates insulin secretion (see below).

Plasma glucose flows down its concentration gradient into the β cell via a specific plasma membrane transporter, **GLUT2.** In the presence of high plasma levels of glucose (e.g., in the fed state), more glucose flows into the cell, where it is phosphorylated to glucose-6-phosphate by hexokinase and, thus, started down the glycolytic pathway. The metabolism of glucose in glycolysis and the citric acid cycle generates ATP, increasing the ATP/ADP ratio in the β cell. The ATP/ADP ratio modulates the activity of a membrane-spanning **ATP-sensitive $K^+$ channel ($K^+$/ATP channel).** *When open, this channel hyperpolarizes the cell by allowing outward flux of $K^+$; when closed, the cell depolarizes.* Because ATP inhibits the channel and ADP activates the channel, a high intracellular ATP/ADP ratio closes the $K^+$/ATP channel. The resulting depolarization of the cell activates voltage-gated $Ca^{2+}$ channels, which mediate an influx of extracellular $Ca^{2+}$. The increase in intracellular [$Ca^{2+}$] stimulates exocytosis of the insulin-containing vesicles. In contrast, under conditions of relatively low extracellular glucose concentrations (e.g., in the fasting state), the β cell has a low ATP/ADP ratio. In this case, the $K^+$/ATP channels remain open, and the β cell is maintained in a hyperpolarized state that prevents $Ca^{2+}$ influx and insulin secretion (Fig. 28-3).

$K^+$/ATP channels are octameric structures containing 4 subunits of Kir6.x and 4 subunits of SURx, where "x" denotes one of several isoforms. The Kir6.x tetramer forms the pore of the $K^+$/ATP channel, while the associated SUR molecules regulate the channel's sensitivity to ADP and pharmacologic agents. Both Kir and SUR subunits must be expressed for a functional channel to be inserted into the plasma membrane. The pancreatic β cell channel is composed of Kir6.2 and SUR1 subunits. $K^+$/ATP channels composed of Kir6.2 and SUR1 isoforms are also expressed in some neurons, while the channels in cardiac and smooth muscle contain SUR2 isoforms. In addition, some smooth muscle cell channels contain Kir6.1 in place of Kir6.2. Mutations in Kir6.2 or SUR1 can result in hyperinsulinemic hypoglycemia, because the β cell is continually depolarized in the absence of $K^+$/ATP channel activity. In the future, elucidation of the mechanisms that regulate the tissue-specific expression of the different Kir6 and SUR isoforms could lead to the development of more specific pharmacologic agents for the treatment of Type II diabetes mellitus.

Kir6.2 binds ATP directly (although the relevant nucleotide-binding motifs have not been identified), and this ATP binding inhibits the $K^+$ conductance of the channel. SUR1 enhances the sensitivity of the Kir6.2 channel to ATP; SUR1 also confers

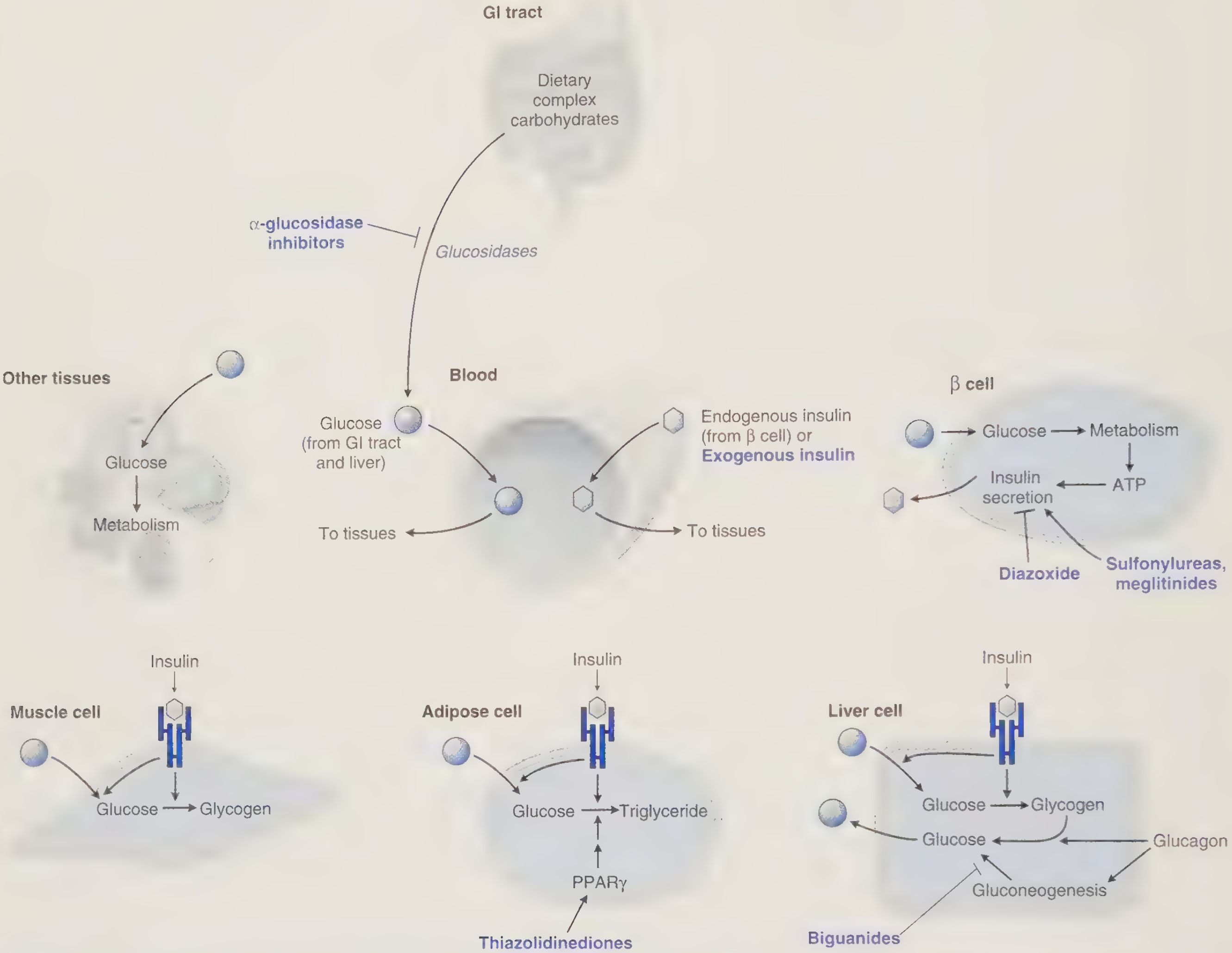

GI tract
Dietary complex carbohydrates
α-glucosidase inhibitors
Glucosidases
Other tissues
Glucose
Metabolism
Blood
Glucose (from GI tract and liver)
To tissues
Endogenous insulin (from β cell) or
Exogenous insulin
To tissues
β cell
Glucose
Metabolism
ATP
Insulin secretion
Diazoxide
Sulfonylureas, meglitinides
Insulin
Muscle cell
Glucose
Glycogen
Insulin
Adipose cell
Glucose
Triglyceride
PPARγ
Thiazolidinediones
Insulin
Liver cell
Glucose
Glycogen
Glucose
Glucagon
Gluconeogenesis
Biguanides

**Figure 28-1. Physiologic and Pharmacologic Regulation of Glucose Homeostasis.** Dietary complex carbohydrates are broken down to simple sugars in the GI tract by the action of glucosidases; simple sugars are then absorbed by GI epithelial cells and transported into the bloodstream. Glucose in the blood is taken up by all metabolically active tissues in the body. In pancreatic β cells, glucose metabolism increases levels of cytosolic ATP, which stimulates insulin secretion. Insulin then acts on plasma membrane insulin receptors in target tissues (muscle, adipose, liver) to increase glucose uptake and storage as glycogen or triglyceride. Glucose is also taken up by other cells and tissues to fuel metabolism. In muscle cells, insulin promotes glucose storage as glycogen. In adipose cells, insulin promotes glucose conversion to triglycerides. Peroxisome proliferator-activated receptor γ (PPARγ) also promotes the conversion of glucose to triglycerides in adipose cells. In liver cells, insulin promotes glucose storage as glycogen. Glucagon promotes both the conversion of glycogen back to glucose and gluconeogenesis; glucose generated from glycogen or by gluconeogenesis is transported out of the liver cell into the bloodstream. Note that glucose from dietary complex carbohydrates, and insulin secreted by pancreatic β cells, both enter the liver in high concentrations through the portal circulation (not shown). Pharmacologic interventions that decrease blood glucose levels include: inhibiting intestinal α-glucosidases; administering exogenous insulin; using sulfonylureas or meglitinides to augment secretion of insulin by β cells; and using biguanides or thiazolidinediones to enhance the action of insulin in liver or adipose cells, respectively. Diazoxide inhibits insulin secretion from pancreatic β cells.

sensitivity of the channel to ADP and to most of the drugs that regulate the activity of the $K^+$/ATP channel. SUR1 contains two nucleotide-binding folds that coordinate ADP complexed with $Mg^{2+}$ ($Mg^{2+}$-ADP); $Mg^{2+}$-ADP binding to SUR1 activates the channel and, thereby, inhibits insulin secretion when the ATP/ADP ratio is low.

Other than plasma glucose, the stimulators of insulin release include several fuels that act to increase the ATP/ADP ratio, including some nonglucose sugars, amino acids, and fatty acids. Parasympathetic nervous system activity and the gastrointestinal hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) also decrease $K^+$/ATP channel activity via G protein-mediated pathways. β Cell exposure to nutrients stimulates not only insulin secretion, but also insulin transcription, translation, processing, and packaging.

## Action at Target Tissues

Insulin binds to receptors on the surface of target cells. Although virtually all tissues express **insulin receptors,** the energy-storing tissues (liver, muscle, and adipose) express

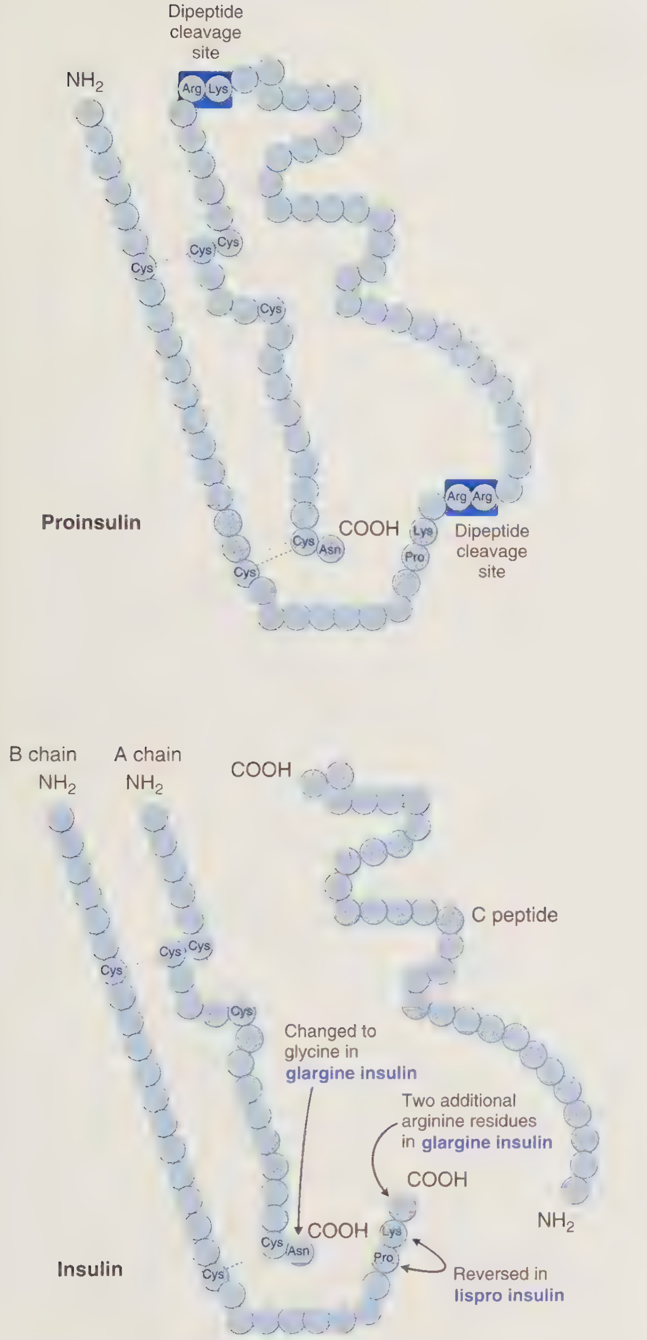

Dipeptide cleavage site
$NH_2$
Arg
Lys
Cys
Cys
Cys
Cys
Arg
Arg
Proinsulin
COOH
Lys
Cys
Asn
Pro
Dipeptide cleavage site
Cys
B chain
A chain
$NH_2$
$NH_2$
COOH
C peptide
Cys
Cys
Cys
Cys
Changed to glycine in glargine insulin
Two additional arginine residues in glargine insulin
COOH
$NH_2$
COOH
Lys
Cys
Asn
Pro
Insulin
Cys
Reversed in lispro insulin

**Figure 28-2. Processing of Human Insulin.** Preproinsulin is synthesized and exported into the endoplasmic reticulum, where the signal peptide (not shown) is cleaved to generate proinsulin **(top panel).** Intramolecular disulfide bonds (cys–cys) aid in the proper folding of proinsulin. Proinsulin is transported to secretory vesicles, where prohormone convertases act on dipeptide cleavage sites in proinsulin (boxes) to generate insulin and connecting (C) peptide. Two disulfide bonds aid in holding the A-chain and B-chain of insulin together. Insulin and C-peptide are secreted together from the pancreatic β cell **(bottom panel).** In lispro, an artificial insulin designed to be absorbed more rapidly after injection, a proline and a lysine residue in the COOH-terminus of the B-chain of insulin are transposed; this minor alteration does not affect the ability of the molecule to bind the insulin receptor or to mediate insulin action. In glargine insulin, an A-chain asparagine is replaced with glycine, and two arginines are added to the COOH-terminus of the B-chain. These modifications slow the absorption of glargine insulin relative to regular insulin.

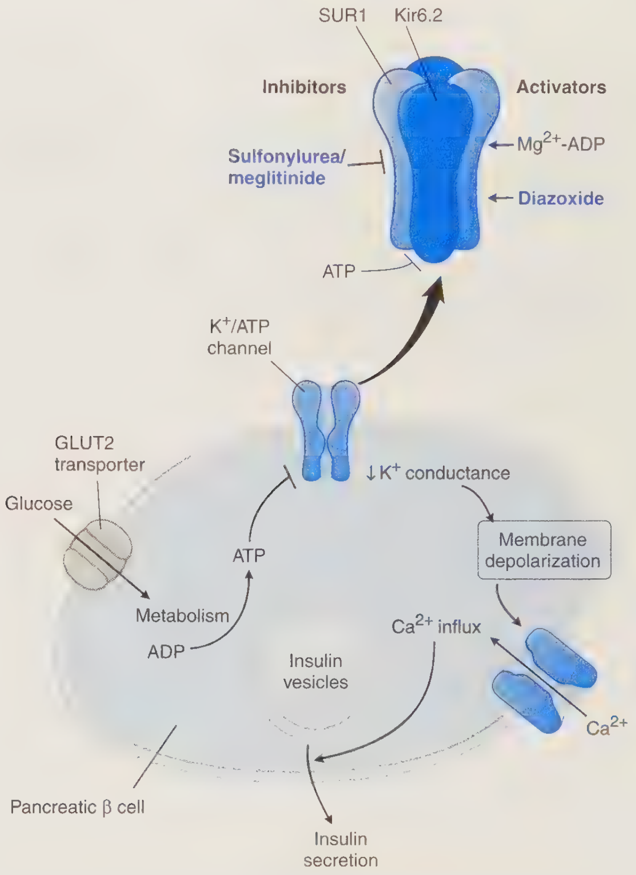

SUR1
Kir6.2
Inhibitors
Activators
Sulfonylurea/ meglitinide
$Mg^{2+}$-ADP
Diazoxide
ATP
$K^+$/ATP channel
GLUT2 transporter
↓$K^+$ conductance
Glucose
Membrane depolarization
ATP
Metabolism
ADP
$Ca^{2+}$ influx
Insulin vesicles
$Ca^{2+}$
Pancreatic β cell
Insulin secretion

**Figure 28-3. Physiologic and Pharmacologic Regulation of Insulin Release from Pancreatic β cells.** In the basal state, the plasma membrane of the β cell is hyperpolarized, and the rate of insulin secretion from the cell is low. When glucose is available, it enters the cell via GLUT2 transporters in the plasma membrane and is metabolized to generate intracellular ATP. ATP binds to and inhibits the plasma membrane $K^+$/ATP channel. Inhibition of the $K^+$/ATP channel decreases plasma membrane $K^+$ conductance; the resulting depolarization of the membrane activates voltage-gated $Ca^{2+}$ channels and, thereby, stimulates an influx of $Ca^{2+}$. $Ca^{2+}$ mediates fusion of insulin-containing secretory vesicles with the plasma membrane, leading to insulin secretion. The $K^+$/ATP channel, an octamer composed of Kir6.2 and SUR1 subunits, is the target of several physiologic and pharmacologic regulators. ATP binds to and inhibits Kir6.2, while sulfonylureas and meglitinides bind to and inhibit SUR1; all three of these agents promote insulin secretion. $Mg^{2+}$-ADP and diazoxide bind to and activate SUR1, thereby inhibiting insulin secretion. (For clarity, only four of the eight $K^+$/ATP channel subunits are shown.)

much higher levels of insulin receptors and, thus, constitute the main insulin target tissues. The insulin receptor (Fig. 28-4) is a glycoprotein consisting of four disulfide-linked subunits, including two extracellular α subunits and two β subunits. Each of the β subunits is composed of a short extracellular domain, a transmembrane domain, and an intracellular tail that contains a tyrosine kinase domain. The binding of insulin to the extracellular portion of the insulin receptor activates the intracellular tyrosine kinase, resulting in "autophosphorylation" of tyrosine on the nearby β subunit and in the phosphorylation of several other intracellular proteins—including **insulin receptor substrate-proteins (IRS-proteins).** Tyrosine-phosphorylated IRS-proteins

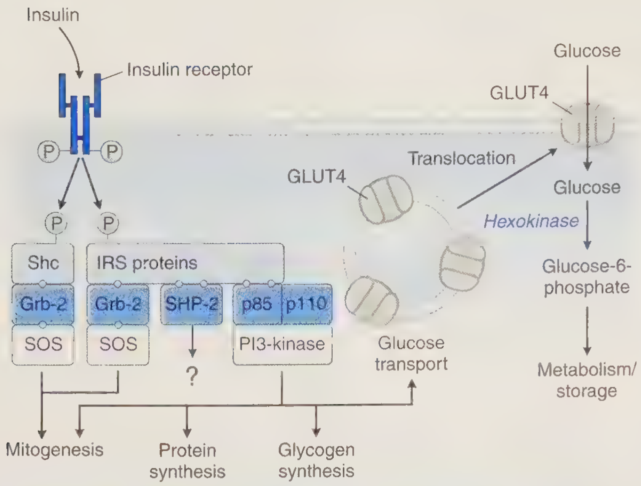


**Figure 28-4. Downstream Effects of Insulin Receptor Activation.** The insulin receptor is a cell surface heterotetramer composed of two α-subunits and two β-subunits. The α-subunits are entirely extracellular, while the β-subunits contain extracellular, transmembrane, and intracellular domains. Insulin binding to the extracellular portion of the receptor activates tyrosine kinase domains in the intracellular regions of the β-subunits. These tyrosine kinase domains mediate "autophosphorylation" of the receptor (actually, each β-subunit phosphorylates the other) and tyrosine-phosphorylation of cytoplasmic substrate proteins including Shc and insulin receptor substrate (IRS) proteins. Phosphorylated Shc promotes mitogenesis. Phosphorylated IRS proteins interact with many other signaling proteins (Grb-2, SHP-2, p85, and p110) to effect changes in cellular function. The IRS interaction with p85 and p110 recruits phosphatidylinositol 3′-kinase (PI3-kinase). PI3-kinase activates signaling cascades that control many aspects of cellular insulin action, including glucose transport (via the translocation of GLUT4 glucose transporters to the cell surface), protein synthesis, and glycogen synthesis. Glucose that enters the cell is rapidly phosphorylated by hexokinase, and subsequently used for metabolism or stored in the cell as glycogen or triglyceride.

recruit a variety of second messenger proteins that contain phosphotyrosine-binding src homology 2 (SH2) domains. The Type IA phosphatidylinositol 3′-kinase is one of these second messenger proteins that appears to be important for many aspects of insulin action.

Although the details linking these insulin receptor second messengers to the metabolic effects of insulin remain open to investigation, the metabolic effects of insulin action are well understood: *insulin is the classic anabolic (energy-storing) hormone.* In the liver, insulin increases glucokinase activity, thereby mediating the phosphorylation and trapping of glucose in hepatocytes. This increased supply of glucose in the hepatocyte provides fuel for glycogen synthesis, glycolysis, and fatty acid synthesis. Insulin's activation of glycogen and fatty acid synthases, and its inhibition of glycogen phosphorylase and gluconeogenic enzymes, combine to further enhance the anabolic processes.

In skeletal muscle and adipose tissue, insulin stimulates the translocation of the insulin-responsive glucose transporter, GLUT4, from intracellular vesicles to the cell surface. to GLUT4 translocation, in turn, facilitates the movement of glucose into the cell. In muscle, insulin also increases amino acid uptake, stimulates the ribosomal protein synthesis machinery, and promotes glycogen synthase activity and subsequent glycogen storage. In adipose tissue, insulin also promotes the expression of lipoprotein lipase, which hydrolyzes triglycerides from circulating lipoproteins for uptake into fat cells. Once inside the fat cell, glucose and fatty acids are stored predominantly as triglycerides. This process is enhanced by the activation of other lipogenic enzymes, including pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase, and glycerol phosphate acyltransferase, and the deactivation of hormone sensitive lipase, which degrades triglyceride. Insulin is degraded rapidly by insulinase enzymes in the liver and kidney; its circulating half-life is 6 minutes.

## GLUCAGON

Glucagon—a single chain protein of 29 amino acids—is a catabolic (energy-releasing) hormone secreted by pancreatic α cells. When plasma glucose levels are low, glucagon mobilizes glucose, fat, and protein from storage for use as sources of energy. Besides low glucose and high insulin levels, stimuli for glucagon secretion include sympathetic nervous system activity, stress, exercise, and high plasma levels of amino acids (because the latter indicates a state of starvation). The binding of glucagon to its G protein-coupled receptor on the plasma membrane of target cells increases intracellular cAMP and activates protein kinase A, a serine/threonine kinase. Glucagon's main site of action is the liver, where it promotes glycogenolysis and gluconeogenesis. Glucagon also promotes lipolysis in adipose tissue. The liver and kidneys degrade glucagon; like insulin, its circulating half-life is about 6 minutes.

## SOMATOSTATIN

Somatostatin—a 14-amino-acid peptide—is produced in multiple sites, including pancreatic δ cells, the gastrointestinal tract, and the hypothalamus. Somatostatin has several inhibitory effects. For example, it decreases secretion of both insulin and glucagon. Somatostatin also inhibits GI tract motility and the secretion of thyroid stimulating hormone, growth hormone, and various gastrointestinal hormones. The stimuli for somatostatin release are similar to those for insulin release; that is, high plasma levels of glucose, amino acids, and fatty acids. Local somatostatin release allows the hormone to act in a paracrine fashion. The circulating half-life of somatostatin is only 2 minutes.

# PATHOPHYSIOLOGY

## DIABETES MELLITUS

As early as AD 200, the Greek physician Aretaeus observed patients who had excessive thirst and urination. He named this condition "diabetes," which is Greek for "to siphon, or pass through." Later, physicians added "mellitus" (Latin for "honeyed, sweet") to the disease name after noticing that diabetic patients produce urine that contains sugar. The designation *diabetes mellitus* also distinguishes this disorder from *diabetes insipidus* (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland), in which dysregulation of the antidiuretic hormone (ADH) response inhibits reabsorption of water in the collecting ducts of the nephron, resulting in the production of copious amounts of dilute urine.

*The syndrome of diabetes mellitus results from a heterogeneous group of metabolic disorders characterized by hyperglycemia* (Table 28-2). Hyperglycemia can result from an absolute lack of insulin [**Type I diabetes mellitus,** also called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes] or from a relative insufficiency of insulin production in the face of insulin resistance [**Type II diabetes mellitus,** also called noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes].

## Type I Diabetes

Type I diabetes mellitus, which accounts for approximately 10% of diabetes in the United States, results from the autoimmune destruction of pancreatic β cells. In the absence of β cells, insulin is neither produced nor released, and circulating insulin concentrations fall to zero. In the absence of insulin, insulin-responsive tissues fail to take up and store glucose, amino acids, and lipids, even when there are high circulating plasma levels of these fuels. *The unopposed action of the counter-regulatory hormones induces a typical starvation response by the cells and tissues of the body.* Thus, glycogenolysis and gluconeogenesis proceed unchecked in the liver, delivering glucose to the circulation even though the blood glucose concentrations are high. Muscle tissue breaks down protein and releases amino acids, which travel to the liver as fuel for gluconeogenesis. In adipose tissue, triglycerides are broken down and released into the circulation. In addition, the liver breaks down fatty acids for use as gluconeogenic fuels and for export as ketone bodies that could be used as fuel by the brain. These ketones equilibrate into β-hydroxybutyrate and acetoacetate. Excessively high concentrations of these acids can deplete serum bicarbonate, eventually resulting in a state of metabolic acidosis called **diabetic ketoacidosis (DKA).** DKA is a potentially life-threatening condition if left untreated.

Blood glucose levels in Type I diabetics exceed the kidney's capacity to reabsorb glucose from the glomerular filtrate, and the glucose that remains in the urine produces an osmotic diuresis as well as a "sweetening" of the urine. This phenomenon causes the polyuria and subsequent polydipsia that many diabetic patients experience. Although appetite is stimulated—resulting in excessive hunger, or polyphagia—patients lose weight because dietary nutrients cannot be stored.

The onset of clinical disease is usually sudden, and often occurs during childhood or adolescence. The actual destruction of β cells occurs gradually, but the remaining β cells can provide a sufficient amount of insulin until approximately 85% of the total population of β cells is destroyed—resulting in the abrupt onset of symptoms. Because 15% of the β cells remain at this point in time, many patients experience a "honeymoon" phase of their illness, with intermittent periods of adequate

**TABLE 28-2** Type I and Type II Diabetes Mellitus

| | TYPE I | TYPE II |
|---|---|---|
| Etiology | Autoimmune destruction of pancreatic β cells | Insulin resistance, with inadequate β cell function to compensate |
| Insulin levels | Zero | Typically higher than normal |
| Insulin action | Zero | Decreased |
| Insulin resistance | Not part of syndrome, but may be present (e.g., in obese patients) | Yes |
| Age of onset | Typically <30 years | Typically >40 years |
| Acute complications | Ketoacidosis<br>Wasting | Hyperglycemia (can lead to hyperosmotic seizures and coma) |
| Chronic complications | Neuropathy<br>Retinopathy<br>Nephropathy<br>Peripheral vascular disease<br>Coronary artery disease | Same as Type I |
| Pharmacologic interventions | Insulin | A number of drug classes are available, including insulin if other therapies fail. |

Type I and Type II diabetes mellitus are both associated with increased blood glucose levels, but the two diseases result from distinct pathophysiologic pathways. In Type I diabetes mellitus, there is an absolute lack of insulin secondary to autoimmune destruction of pancreatic β cells. The etiology of Type II diabetes is less well understood, but seems to involve impaired insulin sensitivity and an inadequate level of compensatory insulin production by pancreatic β cells. Although Type I and Type II diabetes have different acute complications (see text), they share similar chronic complications. Insulin is the primary pharmacologic intervention for Type I diabetes, while Type II diabetes can be treated with a number of different agents.

endogenous insulin production before the eventual complete and final loss of insulin production. In many cases, a prodromal "flu-like" syndrome occurs a few weeks before the onset of symptomatic diabetes. Although some hypotheses suggest that this syndrome represents a viral illness that triggers an autoimmune reaction in genetically predisposed individuals, it is possible that these patients are instead reacting to increased levels of inflammatory mediators produced by an already-initiated autoimmune reaction.

The genetic predisposition to Type I diabetes maps most tightly to certain alleles on chromosome 6. These alleles code for human leukocyte antigens (HLA), also called major histocompatibility complex (MHC) proteins, that are involved in antigen presentation in the immune system. Other genetic loci may also contribute to the development of Type I diabetes. In most Type I patients, autoantibodies to β cell proteins can be detected. Environmental factors influence disease development as well; if one member of an identical twin pair is affected, the incidence of Type I diabetes in the other twin is about 50%.

Because Type I patients produce no endogenous insulin, therapy consists of replacement with exogenous insulin (see below).

## Type II Diabetes

Type II diabetes mellitus, which constitutes approximately 90% of diabetes in the United States, typically affects individuals over 40 years old. Obesity is the single most important risk factor for Type II diabetes; 80% of all Type II diabetic patients are obese. The disorder typically develops gradually, without obvious symptoms at the onset and is frequently diagnosed either by elevated blood glucose in routine screening tests or after the disease has become severe enough to cause polyuria and polydipsia, as in the introductory case.

The progression to Type II diabetes is thought to begin with a state of **insulin resistance.** Tissues that were once normally insulin-responsive become relatively refractory to insulin action, and require increased insulin levels to respond appropriately. In many cases, insulin resistance is the result of obesity and a sedentary lifestyle, although the molecular predisposition in these patients is poorly characterized. Many investigators have described insulin receptor as well as postreceptor signaling defects. However, it is unclear which, if any, of these defects could be the primary event in insulin resistance. Initially, insulin resistance is compensated for by increased production of insulin by pancreatic β cells. Indeed, many individuals with obesity and insulin resistance never progress to frank diabetes, because their β cells continue to compensate by increased secretion of insulin. In some patients like Mrs. Sweetie, however, the β cells eventually fail to keep pace with the demand for insulin.

Although patients with Type II diabetes generally have increased circulating insulin levels, these levels are insufficient to overcome the target tissue insulin resistance. The eventual failure of β cell compensation could result from the loss of β cells through increased apoptosis (programmed cell death) or from the decreased renewal of β cells. Insulin levels that are inadequate to compensate for insulin resistance stimulate an inadequate response in target tissues, resulting in an imbalance between the actions of insulin and the counter-regulatory hormones. This imbalance leads to hyperglycemia and dyslipidemia, as the liver and adipose tissues inappropriately mobilize fuels from storage (see above).

The genetic basis for Type II diabetes is likely a combination of predispositions toward obesity, insulin resistance, and β cell failure. Type II diabetic patients who are lean (and insulin-sensitive) generally have a strong predisposition to β cell failure. Indeed, an early and extreme form of Type II diabetes—mature onset diabetes in the young (MODY)—results from a predisposition to early β cell failure; in many cases, the molecular basis for this predisposition is an inherited mutation in one of the β cell-specific transcription factors. Mild or early Type II diabetes can be unmasked in predisposed individuals by states in which insulin resistance occurs suddenly, such as treatment with glucocorticoids (see Chapter 26, Pharmacology of the Adrenal Cortex) or pregnancy (gestational diabetes).

There does not seem to be an autoimmune contribution to the development of Type II diabetes, although there are rare syndromes of insulin resistance associated with autoantibodies directed against insulin or the insulin receptor. Other rare mutations in the insulin receptor can also lead to severe insulin resistance. In some cases, these individuals never progress to frank diabetes, because their β cells are able to compensate by overproducing insulin.

The ability of patients with Type II diabetes (like Mrs. Sweetie) to produce some insulin suggests that such patients can be treated with orally available agents that: (1) control blood glucose levels by slowing the absorption of sugars from the GI tract; (2) increase insulin secretion by pancreatic β cells, or; (3) sensitize target cells to the action of insulin. Type II diabetic patients who have lost a great deal of β cell function can come to resemble Type I diabetic patients clinically; many such patients eventually require exogenous insulin therapy.

## Morbidity and Mortality

Type I and Type II diabetes each have acute and chronic morbidities. In uncontrolled Type I diabetes, the unopposed action of counter-regulatory hormones leads to ketoacidosis, which can progress rapidly to coma and death. Even in the absence of ketoacidosis, the lack of insulin leads to wasting and death over a period of weeks to months. Ketoacidosis does not generally occur in Type II diabetes, because some endogenous insulin remains. However, extreme hyperglycemia in Type I or Type II diabetes can cause a hyperosmotic syndrome that leads to mental status changes and can progress to seizures, coma, and death. Both Type I and Type II diabetes are associated with long-term vascular pathology. These **chronic complications** include premature atherosclerosis, retinopathy, nephropathy, and neuropathy. Although the exact mechanisms are unclear, it seems that these complications result from hyperglycemia, and perhaps hyperlipidemia, over many years. In treating Mrs. Sweetie's diabetes, the goals are not only to improve her polydipsia and polyuria and to normalize her laboratory values as ends in themselves, but also to prevent these serious chronic complications.

Because uncontrolled diabetes has such severe complications, it is critical to assess accurately the level of control achieved with any therapy. The results of the landmark Diabetes Control and Complications Trial (DCCT), a multicenter clinical trial (1983–1996) involving Type I diabetic patients, and the United Kingdom Prospective Diabetes Study (UKPDS, 1998) involving Type II diabetics, suggest that *intensive therapy to maintain continual normoglycemia dramatically reduces the incidence of the long-term complications of diabetes.*

Blood glucose levels are assessed in two ways: acutely, by measuring blood glucose with a glucose monitor, and chronically, by measuring **glycohemoglobin (HbA1c).** "Tight control," or the maintenance of normoglycemia, is generally achieved by measuring acute blood glucose levels several times throughout the day, and altering diet and insulin doses to keep blood glucose levels within the normal range. To obtain an estimate of the average blood glucose level over the previous several months, physicians can measure HbA1c. Glucose in the blood nonenzymatically glycosylates blood proteins; the nonenzymatic glycosylation of hemoglobin in red blood cells generates HbA1c. Because nonenzymatic glycosylation occurs at a rate that is proportional to the level of glucose in the blood, and the lifespan of a red blood cell is approximately 120 days, the HbA1c level yields an estimate of the average blood glucose level for the preceding several months. Consequently, the HbA1c value can be elevated in a patient at the same time that the blood glucose level is normal—meaning that, although the blood glucose level is acutely normal, glucose levels have been chronically elevated over the previous several months. Mrs. Sweetie's HbA1c level of 9.2% is cause for concern because the rate of chronic diabetic complications rises dramatically with HbA1c levels greater than 7.5%. HbA1c levels may be misleadingly low in patients with shortened red blood cell lifespans (e.g., in patients with hemolytic anemia).

## HYPERINSULINEMIA

Hyperinsulinemia is one of several conditions that can result in hypoglycemia. Hypoglycemia is a problematic condition because the brain requires a constant supply of glucose and cannot rely on alternate fuels as readily as peripheral tissues can. Hyperinsulinemia has various causes, the most common of which is iatrogenic (i.e., exogenous insulin overdose in the course of insulin therapy for Type I or Type II diabetes). A central challenge in the therapy of diabetes (Type I or Type II) is to normalize glucose levels adequately while avoiding overtreatment and causing hypoglycemia. Other rare causes of hypoglycemia include insulinomas (insulin-secreting tumors of pancreatic β cells), mutations in the β cell $K^+$/ATP channel (e.g., mutations in Kir6.2 or SUR1 that result in constitutive depolarization, as described above), and activating autoantibodies directed against the insulin receptor.

# PHARMACOLOGIC CLASSES AND AGENTS

## THERAPY FOR DIABETES

### Strategies of Therapy

A major goal of pharmacologic therapy for diabetes is to normalize blood sugar to reduce the risk of long-term complications. For Type I diabetic patients, the pharmacologic strategy is to administer a sufficient amount of exogenous insulin to achieve normoglycemia, without inducing hypoglycemia. Appropriate treatment of Type I diabetic patients not only achieves normoglycemia, but also reverses the metabolic starvation response mediated by the unopposed action of counter-regulatory hormones; for example, insulin reverses amino acid breakdown in muscle and ketogenesis in the liver.

The treatment of Type II diabetes is multifaceted. First, obese patients should endeavor to reduce body weight and increase exercise to improve insulin sensitivity. Some Type II patients can achieve good control of their diabetes by modifying their diet and exercise habits; Mrs. Sweetie's diabetes would likely be improved dramatically by such lifestyle changes. Pharmacologically, treatments include orally available agents that act to slow glucose absorption from the gut (α-glucosidase inhibitors), to increase insulin secretion by β cells (sulfonylureas and meglitinides), or to increase insulin sensitivity at target tissues (thiazolidinediones and biguanides). All of these agents are ineffective for patients with Type I diabetes. Patients with Type II diabetes are frequently treated with combinations of these drugs and, therefore, are utilizing multiple strategies; nonetheless, some will eventually require exogenous insulin therapy. Until 1994, insulin secretagogues were the only oral treatments for Type II diabetes available in the United States. The various agents are discussed below in a framework that highlights their sites and mechanisms of action, by following the path of glucose metabolism from intestinal glucose absorption to insulin secretion to the metabolism and storage of glucose in target tissues (Fig. 28-1).

### Inhibitors of Intestinal Glucose Absorption: α-Glucosidase Inhibitors

**α-Glucosidase inhibitors**—nicknamed "starch blockers"—are carbohydrate analogues that bind 1,000 times more avidly than dietary carbohydrates to intestinal brush border α-glucosidase enzymes. Glucosidases—maltase, isomaltase, sucrase, and glucoamylase—aid absorption by cleaving complex carbohydrates to yield glucose. By reversibly inhibiting these enzymes, α-glucosidase inhibitors increase the time required for absorption of such carbohydrates as starch, dextrin, and disaccharides. These drugs also increase the intestinal surface area for absorption because carbohydrates that would have been absorbed in the upper intestine are absorbed instead—in smaller quantities—throughout the length of the small intestine. Therefore, these drugs help reduce the postprandial peak in blood sugar and are effective when taken with meals; they are not effective for the treatment of hyperglycemia at other times. Mrs. Sweetie's increased *fasting* blood glucose level suggests that, in her case, monotherapy with an α-glucosidase inhibitor would likely be ineffective.

**Acarbose** was introduced in the United States in 1996, and **miglitol** was introduced in 1999; these two agents are similarly effective. When used as monotherapy, these agents reduce fasting plasma glucose by 25–30 mg/dL (1.3–1.7 mmol/L), postprandial plasma glucose by 40–50 mg/dL (2.2–2.8 mmol/L), and HbA1c by 0.7%–0.9%, and they pose no risk of hypoglycemia. α-Glucosidase inhibitors are also useful as adjunctive therapy. These drugs are most useful for patients with predominantly postprandial hyperglycemia, and for new-onset patients with mild hyperglycemia. Flatulence, bloating, abdominal discomfort, and diarrhea are common adverse effects, all of which result from gas released by bacteria acting on undigested carbohydrates that reach the large intestine. The gastrointestinal distress usually diminishes with continued use of an α-glucosidase inhibitor, but these agents are contraindicated for patients with inflammatory bowel disease. Serum aminotransferase levels should be monitored during therapy because these drugs are associated with a dose-dependent increase in serum aminotransferase levels that is reversible upon drug discontinuation. In addition, α-glucosidase inhibitors are associated with modest increases in plasma triglycerides. Use of these agents is not associated with any change in weight.

## Insulin Replacement: Exogenous Insulin

Insulin is the only treatment for patients with Type I diabetes. Insulin is also used for Type II diabetic patients if diet and other therapies are not effective at controlling the hyperglycemia. Insulin preparations are classified according to onset of action, duration of action, and species of origin (i.e., human, pig, or cow). Recombinant DNA techniques have been employed to produce human insulin in vitro, making this form of the drug an increasingly popular choice relative to other (porcine or bovine) preparations that can violate certain religious restrictions and/or provoke an immune response.

*Because insulin is a protein that would be subject to rapid degradation in the GI tract, it is not effective as an oral agent.* Instead, insulin is administered by subcutaneous injection with a fine-gauge needle, creating a small depot of insulin at the site of injection. The rate at which this depot of insulin is absorbed depends on a variety of factors, including the solubility of the insulin preparation and the local circulation. The more quickly a particular preparation is absorbed, the faster its onset of action, and the shorter its duration of action. Person-to-person and site-to-site variability can produce great differences in the rate of absorption and, thus, the action profile of the injected insulin. Table 28-3 divides the most commonly used insulin preparations into four categories, based on their onset, peak, and duration of action.

***Regular* insulin,** a short-acting preparation, is structurally identical to endogenous insulin, but it has additional zinc ions added for stability. Regular insulin tends to aggregate into hexamers; dissociation of the hexamers to monomers is the rate-limiting step for absorption. ***Lispro* insulin,** an ultrarapid-acting insulin, was designed to keep the molecule in a monomeric form to speed absorption. Lispro insulin is structurally similar to regular insulin, except that a sequence of two amino acids (lysine and proline) near the carboxy-terminus of the B-chain has been switched (see Fig. 28-2). Lispro offers flexibility and convenience for patients because it

**TABLE 28-3** Commonly Used Insulin Preparations

| TYPE AND PREPARATION | CONSTITUENTS | Action Profile (Hours) | | | USAGE |
|---|---|---|---|---|---|
| | | ONSET | PEAK | DURATION | |
| **Ultrarapid-Acting** | | | | | |
| Lispro (human analogue) | Identical to regular human insulin, except for transposed lysine and proline in B chain | 0.2–0.5 | 0.5–2 | 3–4 | For meals or acute hyperglycemia |
| **Short-Acting** | | | | | |
| Regular (human) | Solution of unmodified zinc insulin crystals | 0.5–1 | 2–3 | 6–8 | For meals or acute hyperglycemia |
| Semilente (human) | Semicrystalline (amorphous) suspension | 1–2 | 2–5 | 8–12 | |
| **Intermediate-Acting** | | | | | |
| NPH (human) | Protamine zinc, phosphate buffer | 1.5 | 4–10 | 16–24 | Provide basal insulin and overnight coverage |
| Lente (human) | Crystalline/amorphous mix, acetate buffer | 1.5–3 | 7–15 | 16–24 | |
| **Long-Acting** | | | | | |
| Ultralente (human) | Crystalline suspension, acetate buffer | 4–6 | 8–30 | 24–36 | Provide basal insulin and overnight coverage |
| Glargine (human analogue) | Similar to regular human insulin, with glycine in place of asparagine in A chain, and 2 extra arginines in B chain | 4–6 | None | 18–24 | |

Modifications to native human insulin consist of either: (1) alterations in the amino acid sequence of the molecule or: (2) changes in the physical form of the molecule. These changes affect the rate at which insulin is absorbed and the temporal profile of insulin action. Alterations in the amino acid sequence change the tendency for insulin to aggregate. The modification in lispro decreases aggregation, resulting in faster absorption and more rapid action. In contrast, a crystalline suspension (ultralente) delays the rate of absorption of insulin from its subcutaneous injection site, making this preparation a long-acting dosage form.

can be injected minutes before a meal; whereas, the proper use of longer-acting insulins requires a time lag between insulin injection and the consumption of a meal. ***NPH* (neutral protamine Hagedorn) insulin,** an intermediate-acting preparation, combines insulin with protamine—a protein isolated from rainbow trout sperm—in a zinc suspension. Protamine prolongs the time required for absorption of the insulin because it complexes with the insulin until proteolytic enzymes cleave the protamine from the insulin. ***Ultralente* insulin,** a long-acting preparation, is a crystalline suspension of insulin and zinc in an acetate buffer. This formulation delays the onset of action of the insulin. ***Semilente* insulin** is semicrystalline, or "amorphous," and is short-acting. ***Lente* insulin** is a combination of crystalline (i.e., ultralente) and semicrystalline (i.e., semilente) insulin and zinc suspended in an acetate buffer. This formulation is slower acting than semilente but faster acting than ultralente, and is, therefore, in the intermediate-acting category. ***Glargine* insulin** is regular insulin in which a glycine replaces an asparagine on the A-chain, and two additional arginines are added at the carboxy terminus of the B-chain (Fig. 28-2). These modifications make the pKa of the insulin more neutral, thus slowing its absorption into the neutral environment of the blood. Glargine has the advantage of long-acting, steady release without a peak (mimicking so-called basal insulin secretion).

Insulin regimens, including the preparation, dose, and frequency of the insulin, are tailored for each patient. Furthermore, regimens are often adjusted slightly on a daily basis according to the patient's activity, meal size and content, and blood glucose levels. For example, some patients inject short-acting insulin before meals, and long-acting insulin to provide basal insulin levels through the night. Advances in insulin therapy are still evolving. Pharmaceutical companies continue to design preparations that will more closely mimic physiologic postprandial serum insulin levels. Researchers are also attempting to make longer-lasting preparations with steadier absorption rates. In addition, new drug delivery techniques are being tested to create alternatives to subcutaneous injection (see Chapter 50, Drug Delivery Mechanisms), such as intranasal and pulmonary dosage forms.

*The major danger with insulin therapy is that administration of insulin in the absence of adequate carbohydrate intake can result in hypoglycemia.* Thus, patients—both Type I and Type II diabetics—must be cautioned not to take too much insulin. While tight glycemic control that aims to maintain normoglycemia does decrease the incidence of diabetic complications, it also increases the frequency of hypoglycemic attacks, because it is challenging to maintain the fine balance between insufficient and excessive insulin.

Insulin resistance in Type II diabetic patients, such as Mrs. Sweetie, is typically more severe in muscle and liver than in fat cells. For this reason, insulin preferentially deposits calories in adipose tissue, and insulin therapy in insulin-resistant patients (especially those who are already obese, like Mrs. Sweetie) often results in weight gain.

## Insulin Secretagogues: Sulfonylureas and Meglitinides

### *Sulfonylureas*

Since the 1950s, **sulfonylureas** have been the major oral agents available in the United States for the treatment of Type II diabetes. Sulfonylureas stimulate insulin release from pancreatic β cells, thereby increasing circulating insulin to levels sufficient to overcome the insulin resistance. *At the molecular level, sulfonylureas act by inhibiting the β cell $K^+$/ATP channel at the SUR1 subunit* (Fig. 28-3). Sulfonylureas may act by displacing endogenous $Mg^{2+}$-ADP, which binds to SUR1 and activates the channel. The sulfonylureas used to treat Type II diabetes bind with a higher affinity to SUR1 than to SUR2 isoforms, accounting for their relative β cell specificity. The inhibition of the $K^+$/ATP channel by sulfonylureas is functionally similar to the molecular events induced physiologically in the fed state, in which increased glucose metabolism causes β cell accumulation of intracellular ATP, membrane depolarization, $Ca^{2+}$ influx, fusion of insulin-containing vesicles with the plasma membrane, and insulin secretion.

Sulfonylureas are orally available and are metabolized by the liver. These drugs are generally safe; their major adverse effect is hypoglycemia resulting from oversecretion of insulin. Thus, these medications should be used cautiously in patients who are unable to recognize or respond appropriately to hypoglycemia, such as those with impaired sympathetic function, mental status changes, or advanced age. Studies show that sulfonylurea use is associated with a marginal decrease in lipids. These agents can cause weight gain secondary to increased insulin activity in adipose tissue; this side effect is obviously counterproductive in obese patients like Mrs. Sweetie. Therefore, sulfonylureas are better suited for nonobese patients. Because first-generation sulfonylureas bind with lower affinity to SUR1 than second-generation agents do, first-generation agents must be administered in higher doses to achieve the same degree of glucose-lowering. As effective, safe, and inexpensive (generically available) drugs, sulfonylureas are a mainstay of treatment for Type II diabetes.

### *Meglitinides*

As with sulfonylureas, **meglitinides** stimulate insulin release by binding to SUR1 and inhibiting the β cell $K^+$/ATP channel. Although both sulfonylureas and meglitinides act on the SUR1 subunit, these two classes of drugs bind to distinct regions of the SUR1 molecule. The absorption, metabolism, and side effect profiles for meglitinides are similar to those for sulfonylureas.

## Insulin Sensitizers: Thiazolidinediones and Biguanides

### *Thiazolidinediones*

The **thiazolidinedione (TZD)** drugs are a relatively new class of oral medication for Type II diabetes; the two currently available in the United States—**rosiglitazone** and **pioglitazone**—were FDA-approved for use in 1999. The TZDs do not affect insulin secretion, but rather *enhance the action of insulin at target tissues.* TZDs are agonists for the nuclear hormone receptor **peroxisome proliferator activated receptor-γ (PPARγ).** The identities of the endogenous ligand(s) for PPARγ remain to be elucidated. PPARγ functions as a heterodimer with the retinoid X receptor (RXR, another nuclear hormone receptor isoform) to activate the transcription of a subset of genes; not all of these genes have been positively identified. PPARγ is expressed primarily in adipose tissue and is involved in adipocyte differentiation. Studies show that cells made to overexpress PPARγ accumulate triglyceride and acquire other adipocyte markers when treated with TZDs. Although treatment with TZDs improves insulin sensitivity not only in adipose tissue but also in liver and muscle (the primary sites of insulin resistance in Type II diabetes), the mechanisms responsible for the effects of TZDs in liver and muscle are mysterious, especially because PPARγ is expressed at low levels in these tissues. In fact, the effect of TZDs on liver and muscle is likely to be indirect, because in vitro TZD treatment of these isolated tissues has little effect (except for inhibition of gluconeogenesis in hepatocytes). One theory suggests that TZD/PPARγ-mediated changes in adipocyte gene expression result in changes in fat metabolism that alter the environment of the liver and muscle cells, ultimately increasing the insulin sensitivity of these tissues.

Although our understanding of the molecular mechanisms by which TZDs act remains incomplete, it is clear that TZD treatment does increase insulin sensitivity, leading to lowering of blood glucose and insulin levels. The insulin-sensitizing effects of TZDs are beneficial in treating not only Type II diabetes, but also other insulin resistance/hyperinsulinemia-associated syndromes such as polycystic ovarian syndrome (PCOS; see Chapter 27, Pharmacology of Reproduction).

Because TZDs are a newer class of drugs, their side effects are still being identified. Effects such as weight gain and decreases in circulating triglyceride and free fatty acid levels can be explained by the stimulatory effect of TZDs on adipocytes. Unlike the insulin secretagogues, the TZDs do not increase insulin levels, and, therefore, do not induce hypoglycemia. The original TZD introduced in the United States (troglitazone) was associated with rare hepatotoxicity, resulting in its withdrawal from the market. Newer TZDs seem to have less hepatotoxicity.

### *Biguanides*

Like TZDs, **biguanides** act by increasing insulin sensitivity. The molecular target of the biguanides seems to be the AMP-dependent protein kinase (AMPPK—not to be confused with PKA). Biguanides activate AMPPK to block the breakdown of fatty acids and to inhibit hepatic gluconeogenesis and glycogenolysis. Secondary effects include increased insulin signaling (i.e., increased activity of the insulin receptor) as well as increased metabolic responsiveness by the liver and skeletal muscle. The most common adverse effect is mild gastrointestinal distress, which is usually transient and can be minimized by slow titration of the dose. A potentially more serious adverse effect is **lactic acidosis.** Because biguanides decrease the flow of metabolic acids through gluconeogenic pathways, lactic acid can accumulate to dangerous levels in biguanide-treated patients. With **metformin,** introduced in the United States in 1995 and the only biguanide currently available in the US, the incidence of lactic acidosis is low and predictable. In fact, this side effect is more commonly associated with other conditions that predispose patients to metabolic acidosis. Thus, metformin should not be administered to patients with hepatic disease, congestive heart failure, respiratory disease, hypoxemia, severe infection, alcohol abuse, a tendency to ketoacidosis, or renal disease (the latter because biguanides are excreted by the kidneys).

Like TZDs, biguanides do not directly affect insulin secretion, and their use is not associated with hypoglycemia. Furthermore, unlike insulin and insulin secretagogues, biguanides are associated with a lowering of serum lipids and a decrease in weight. Also like TZDs, biguanides are useful in the treatment of other conditions, such as PCOS, that are associated with insulin resistance and hyperinsulinemia.

## Combination Therapy

As discussed above, insulin-requiring diabetics, including Type I diabetic patients, benefit from optimizing their therapy with combinations of various short-acting and long-acting insulin preparations. As more oral agents for the treatment of Type II diabetes have become available, **oral combination therapy** for Type II patients has also become a reality. *In general, combination therapy with drugs that affect different molecular targets, and that have different mechanisms of actions, has the multiple advantages of improving glycemic control while using lower doses of each drug, and, thus, reducing side effects.* For example, combining an insulin sensitizer (e.g., a TZD or metformin) with insulin or an insulin secretagogue (e.g., a sulfonylurea) can both improve glycemic control in a poorly controlled Type II diabetic patient and lower the dose of each drug required to achieve a therapeutic effect. TZDs and metformin, which are both insulin sensitizers but with different mechanisms of action, can also be used together effectively. However, combining two different insulin secretagogues does not improve therapeutic outcomes.

What then, is the optimal treatment for Mrs. Sweetie, given that there are a number of agents from which to choose? First, it is important (as in all cases of diabetes) to promote weight loss and increased exercise. Type II diabetic patients—especially those who are older and obese—are often best started on an insulin-sensitizing agent that does not predispose to either hypoglycemia or weight gain. Because Mrs. Sweetie does not seem to have renal disease or another contraindication to biguanide treatment, metformin could be a reasonable choice. A TZD would also be a reasonable starting point. If monotherapy with metformin or a TZD did not adequately decrease her blood glucose and HbA1c levels, then a combination of the two could be tried. Alternatively, adding a sulfonylurea to the insulin sensitizer could be a reasonable choice. See Table 28-4 for a comparison of side effects associated with long-term use of several different therapies for Type II diabetes.

## THERAPY FOR HYPERINSULINEMIA

Although surgical excision is ultimately the treatment of choice for insulinomas, **diazoxide** and **octreotide** are two drugs that are often used to stabilize hypoglycemic patients preoperatively. Diazoxide binds to the SUR1 subunit of $K^+$/ATP channels in pancreatic β cells, and stabilizes the ATP-bound (open) state of the channel so that the β cells remain hyperpolarized. A number of $K^+$/ATP channel-openers of this type are known, but most are specific for SUR2

TABLE 28-4 Side Effects over 10 Years of Use: A Comparison of Several Agents Used as Monotherapy for Type II Diabetes Mellitus

| AGENT | INCREASE IN WEIGHT (COMPARED TO DIET THERAPY ALONE), *kg* | SEVERE HYPOGLYCEMIA,* *% OF SUBJECTS* | SYMPTOMATIC HYPOGLYCEMIA,** *% OF SUBJECTS* |
|---|---|---|---|
| Insulin | 4.0 | 2.3 | 36 |
| Sulfonylurea | 2.2 | 0.5 | 14 |
| Biguanide | 0 | 0 | 4 |

Because diabetes is a chronic disease, the long-term implications of therapy are an important therapeutic consideration. Insulin and sulfonylureas are both capable of lowering blood glucose to dangerous levels, while biguanides lack this side effect. In addition, biguanide use is not associated with an increase in body weight, while patients taking insulin or a sulfonylurea tend to gain weight.

*Severe hypoglycemia is defined as hypoglycemia requiring hospitalization or other third-party intervention.

**Symptomatic hypoglycemia is defined as hypoglycemia not requiring hospitalization. (Data from the United Kingdom Prospective Diabetes Study (UKPDS) 1998.)

isoforms and, thus, are not useful to target the SUR1/Kir6.2 channel expressed by pancreatic β cells. Diazoxide binds channels containing either SUR1 or SUR2 isoforms, and is therefore used not only to decrease insulin secretion by pancreatic β cells, but also to hyperpolarize SUR2-containing channels in cardiac and smooth muscle cells and, by maintaining these cells in a more relaxed state, to decrease blood pressure in hypertensive emergencies. In a rare form of genetic hyperinsulinemic hypoglycemia, a mutant SUR1 isoform is relatively insensitive to $Mg^{2+}$-ADP but does respond to diazoxide; in most forms of this disease, however, the mutant channel is not transported to the cell surface, and diazoxide is ineffective.

Octreotide is a somatostatin analogue (see Chapter 24) that is longer acting than endogenous somatostatin. As with somatostatin, this agent blocks hormone release from endocrine-secreting tumors, such as insulinomas, glucagonomas, and thyrotropin-secreting pituitary adenomas.

## GLUCAGON AS THERAPEUTIC AGENT

**Glucagon** is used to treat severe hypoglycemia when oral or intravenous glucose administration is not possible. As with insulin, glucagon is administered by subcutaneous injection. The hyperglycemic action of glucagon is transient and depends on a sufficient hepatic store of glycogen. Glucagon is also used as an intestinal relaxant before radiographic or magnetic resonance imaging of the gastrointestinal tract. The mechanism by which glucagon mediates intestinal relaxation remains uncertain.

## *Conclusion and Future Directions*

The pancreatic hormones insulin, glucagon, and somatostatin are involved in fuel homeostasis. When the levels of these hormones are pathologically altered, an individual can become hyperglycemic (as in diabetes mellitus) or hypoglycemic. Various pharmacologic agents act at several different cellular and molecular sites to normalize blood glucose levels. For example, α-glucosidase inhibitors slow the intestinal absorption of carbohydrates. Exogenous insulin, sulfonylureas, and meglitinides increase insulin levels, while diazoxide reduces insulin levels. Thiazolidinediones and biguanides increase insulin sensitivity at target tissues. Octreotide, a synthetic form of somatostatin, has wide-ranging inhibitory effects on hormone secretion. Exogenous glucagon can be used to increase plasma glucose levels. Future research on the pharmacologic treatment of diabetes will focus on better delineation of the molecular mechanisms of current treatments to understand better the molecular and cellular pathophysiology of Type II diabetes mellitus. This research will include, but not be limited to, further research on the targets of PPARγ action. In addition, clinical studies will continue to refine the role of combination oral therapy for Type II diabetes mellitus, in an attempt to maintain long-term normoglycemia without episodes of hypoglycemia.

## *Suggested Readings*

DeWitt DE, Hirsch IB. Outpatient insulin therapy in Type 1 and Type 2 diabetes mellitus: Scientific review. JAMA 2003;289:2254–2264. (*Reviews currently available insulin preparations and their pharmacodynamic and pharmacokinetic profiles*)

Hardie DG. Minireview: The AMP-activated protein kinase cascade: The key sensor of cellular energy status. Endocrinology 2003;144:5179–5183. (*Reviews function and mechanism of action of probable biguanide target*)

Inzucchi SE. Oral antihyperglycemic therapy for Type 2 diabetes: Scientific review. JAMA 2002;287:360–372. (*Reviews mechanisms of action, metabolic effects and adverse effect profiles of the orally available therapies for Type 2 diabetes*)

Moller DE. New drug targets for Type 2 diabetes and the metabolic syndrome. Nature 2001;414:821–827. (*Identifies future drug targets for Type 2 diabetes based on pathophysiologic mechanisms*)

Nathan DM. Initial management of glycemia in Type 2 diabetes mellitus. N Engl J Med 2002;347:1342–1349. (*Clinically oriented approach to treatment of type 2 diabetes, including diet, exercise, insulin, oral agents, and combination therapy*)

Shuldiner AR, Yang R, Gong DW. Resistin, obesity, and insulin resistance—the emerging role of the adipocyte as an endocrine organ. N Engl J Med 2001;345:1345–1346. (*Brief article and references to reviews outlining potential roles for adipocyte hormones in disease pathophysiology*)

## Drug Summary Table Pharmacology of the Endocrine Pancreas

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ALPHA-GLUCOSIDASE INHIBITORS**<br>*Mechanism—Inhibit intestinal brush border glucosidases to slow absorption of glucose after meals* | | | | |
| **Acarbose**<br>**Miglitol**<br>**Voglibose** | Postprandial hyperglycemia in Type II diabetes | Flatulence<br>Bloating<br>Abdominal discomfort<br>Diarrhea<br>Elevated aminotransaminases<br>Elevated triglyceride levels | Not for Type I diabetes<br>Inflammatory bowel disease | Undigested carbohydrates reach bacteria in large intestine, thus producing gas that contributes to gastrointestinal symptoms<br>Not useful in fasting hyperglycemia |
| **EXOGENOUS INSULIN**<br>*Mechanism—Promotes glucose, amino acid, and triglyceride uptake from blood into cells* | | | | |
| **Lispro ultra-rapid acting**<br>**Regular short-acting**<br>**Semilente short-acting**<br>**NPH intermediate-acting**<br>**Lente intermediate-acting**<br>**Ultralente long-acting**<br>**Glargine long-acting** | Type I diabetes<br>Type II diabetes refractory to other agents<br>Acute hyperkalemia (because insulin closes $K^+$ channel and, therefore, prevents $K^+$ from exiting cells) | Hypoglycemia<br>Weight gain | | Subcutaneous or intramuscular route (not orally because GI tract degrades proteins including insulin) |
| **INSULIN SECRETAGOGUES: SULFONYLUREAS AND MEGLITINIDES**<br>*Mechanism—Close $K^+$/ATP channel and depolarize $\beta$ cell to increase insulin secretion* | | | | |
| *First Generation Sulfonylureas:*<br>**Acetohexamide**<br>**Chlorpropamide**<br>**Tolazamide**<br>**Tolbutamide**<br>*Second Generation Sulfonylureas:*<br>**Glimepiride**<br>**Glipizide**<br>**Glibenclamide (Glyburide)**<br>**Gliclazide**<br>**Gliquidone**<br>*Meglitinides:*<br>**Nateglinide**<br>**Repaglinide** | Type II diabetes | Because this class of drugs increases insulin release, hypoglycemia can result<br>Predispose to weight gain | Not for Type I diabetes | Effective and safe<br>Generics are inexpensive<br>Second generation sulfonylureas bind with higher affinity compared to first generation sulfonylureas<br>Sulfonylureas and meglitinides both bind SUR1 subunit of $K^+$/ATP channel—but at different sites<br>Sulfonylureas and meglitinides have similar effects, absorption, metabolism, and side effects |

| | | | | |
|---|---|---|---|---|
| **INSULIN SENSITIZERS: THIAZOLIDINEDIONES (TZDs)**<br>*Mechanism—Bind PPARγ to enhance insulin action in fat, liver and muscle* | | | | |
| **Pioglitazone**<br>**Rosiglitazone** | Type II diabetes<br>Polycystic ovarian syndrome | Weight gain | Not for Type I diabetes | Troglitazone withdrawn from market due to hepatic toxicity<br>Increase HDL, LDL<br>Decrease circulating triglycerides and free fatty acids |
| **INSULIN SENSITIZERS: BIGUANIDES**<br>*Mechanism—Inhibit hepatic gluconeogenesis and glycogenolysis* | | | | |
| **Metformin** | Type II diabetes<br>Polycystic ovarian syndrome | Gastrointestinal distress<br>Lactic acidosis | Contraindicated for Type I diabetes and for any predisposition to acidosis, including respiratory and renal disease, CHF, and others | No weight gain (slight weight loss)<br>Decrease circulating triglycerides and LDL<br>May slightly increase HDL levels |
| **DIAZOXIDE**<br>*Mechanism—Keeps $K^+$/ATP channel open and hyperpolarizes β cell to decrease insulin secretion* | | | | |
| **Diazoxide** | Hypoglycemia | | | |
| **SOMATOSTATIN ANALOGUES**<br>*Mechanism—Decrease hormone secretion; general inhibitory effects* | | | | |
| **Octreotide** | Hormone-releasing tumors<br>Esophageal varices<br>Hypoglycemia | | | Longer circulating half-life than endogenous somatostatin |
| **EXOGENOUS GLUCAGON**<br>*Mechanism—Mobilizes glucose, fat, and protein from storage* | | | | |
| **Glucagon** | Hypoglycemia<br>Intestinal relaxant | | | |

# 29

# Pharmacology of Bone Mineral Homeostasis

Allen S. Liu, Ariel Weissmann, and Armen H. Tashjian, Jr.

**Introduction**
**Case**
**Physiology of Bone Mineral Homeostasis**
- Structure of Bone
- Mineral Balance
- Regulation of Bone Remodeling
- Hormonal Control of Calcium and Phosphate
    - *Parathyroid Hormone*
    - *Vitamin D*
    - *Calcitonin*
    - *Glucocorticoids, Thyroid Hormone, and Gonadal Steroids*

**Pathophysiology**
- Rickets and Osteomalacia
- Hyperparathyroidism
- Hypoparathyroidism
- Osteoporosis
- Paget's Disease
- Chronic Renal Failure

**Pharmacologic Classes and Agents**
- Antiresorptive Agents
    - *Hormone Replacement Therapy (HRT)*
    - *Selective Estrogen Receptor Modulators*
    - *Bisphosphonates*
    - *Calcitonin*
- Bone Anabolic Agents
    - *Fluoride*
    - *Parathyroid Hormone*
- Oral Calcium
- Vitamin D
- Drugs Affecting Plasma Calcium Concentrations
    - *Hypercalcemia and Its Treatment*
    - *Hypocalcemia and Its Treatment*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

The 206 bones of the human skeleton are far from the lifeless structures they are commonly imagined to be. Bones are remodeled continuously and are involved in many functions besides structural support and protection of internal organs, including hematopoiesis and mineral storage. The focus of this chapter is on the critical role of bone in mineral homeostasis, the process and regulation of bone remodeling, the diseases that can result when the delicate balances of mineral homeostasis and bone remodeling are perturbed, and the pharmacologic therapies employed to treat these conditions. A key concept regarding the pharmacologic agents discussed in this chapter is the distinction between bone antiresorptive agents, which slow bone loss, and bone anabolic agents, which have the potential to increase overall bone mass.

## Case

MS is a 60-year-old Caucasian female who comes to her physician with the recent onset of low back pain that began when she unexpectedly stepped into a pothole. She is otherwise in good health.

Her menstrual periods ceased when she was 54 years old. She had little in the way of postmenopausal symptoms and never took hormone replacement therapy. Menarche was at age 11. She has one child who was born when MS was 38 years old. Her mother died at age 55 with breast cancer and her sister, age 58, was recently diagnosed with breast cancer. The patient is moderately active, playing tennis for 1 hour about once a week. Her father and maternal aunt died in their 60s with coronary artery disease.

On physical examination, she has point tenderness over lumbar vertebra L-1. Her weight is 135 lb., and she is 64 inches tall but believes she has lost some height over the last year. Laboratory studies are all within normal limits. Lateral X-ray of the spine shows a compression fracture of L-1 and generalized osteopenia. Measurement of bone mineral density (BMD) at the spine and hip reveals a value that is 2.6 standard deviations below the healthy peak female value.

Her physician diagnoses postmenopausal osteoporosis and a recent compression fracture of L-1. MS asks her physician to discuss with her the current therapeutic options, and is particularly interested in the potential risks and benefits of each option.

## QUESTIONS

1. Why is MS at particularly high risk for osteoporosis?
2. Is this patient at risk for breast cancer and/or cardiovascular disease? How does this alter the choice of pharmacologic agents that could be prescribed?
3. What are the therapeutic options available for MS? What are the advantages and disadvantages of each option?

## PHYSIOLOGY OF BONE MINERAL HOMEOSTASIS

Specialized cells called osteoblasts and osteoclasts continually remodel the human skeleton in response to mechanical forces and humoral factors. Several of these humoral factors—parathyroid hormone, vitamin D, and calcitonin—control the remodeling of bone for the purpose of maintaining calcium homeostasis. Other hormones, such as glucocorticoids, thyroid hormone, and gonadal steroids, also have important effects on bone integrity. This section reviews the cellular and molecular mechanisms that mediate bone formation and bone resorption and the mechanisms by which hormones (especially parathyroid hormone and vitamin D) maintain plasma calcium levels within a very narrow concentration range.

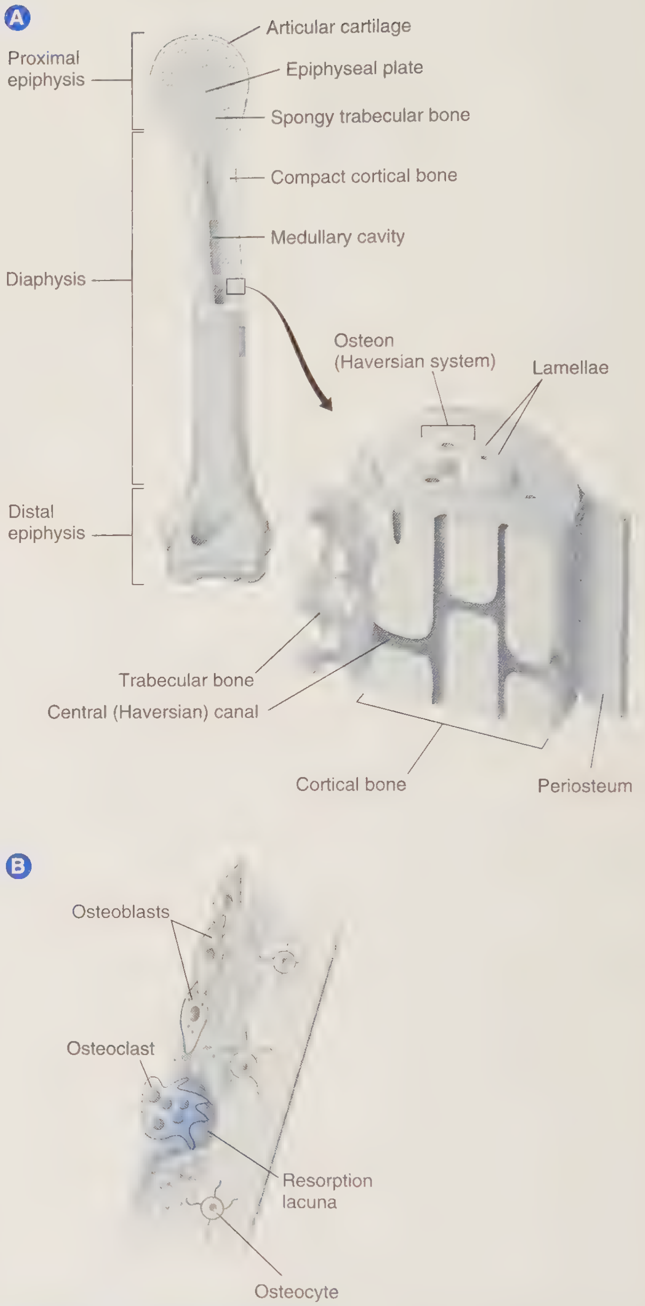


**Figure 29-1. Structure of Bone.** **A,** The upper panel depicts the structure of a long bone (humerus). Note that the diaphysis has a thick shell of compact cortical bone, while the epiphysis is composed predominantly of trabecular bone. The lower panel shows the detailed structure of bone. **B,** Bone remodeling is a dynamic balance between the catabolic activity of osteoclasts and the anabolic activity of osteoblasts. The majority of bone remodeling occurs in trabecular bone. Therefore, any condition that disrupts the process of mineralization and/or bone turnover preferentially affects the skeletal sites and regions of bone that have large trabecular areas. For this reason, osteoporotic fractures occur commonly in vertebral bodies and the neck of the femur.

## STRUCTURE OF BONE

Figure 29-1 illustrates the structure of a long bone. Note that the cortical bone forms a thick outer layer, or "cortex," around a medulla that consists of trabecular bone and bone marrow. In bones such as vertebral bodies, and in the neck and head of the femur, the cortical bone forms a thinner layer surrounding a larger core of trabecular bone. Osteoblasts and osteoclasts are found both on the outer surface of bone (beneath the periosteum) and on all inner surfaces of the bone, including the endosteum that lines the central canals in cortical bone and all of the many surfaces in trabecular bone.

Bone consists of 25% organic and 75% inorganic components. The organic component includes the cells (osteoblasts, osteoclasts, osteocytes, and bone lining cells), osteoid (a matrix consisting primarily of type I collagen fibers), and several other low-abundance proteins. The inorganic component consists of crystalline calcium phosphate salts, primarily **hydroxyapatite.** The chemical formula of hydroxyapatite is $(Ca)_5(PO_4)_3OH$. Ninety-nine percent of the calcium in the body is stored in the skeleton, mostly as hydroxyapatite.

## MINERAL BALANCE

Calcium is absorbed in the small intestine by two mechanisms: facilitated transport, which occurs throughout the small intestine, and vitamin D-dependent active transport, which occurs mainly in the duodenum. Approximately 300 mg, or less than a third of the 1,000-mg average daily intake of dietary calcium, is absorbed by the intestine under normal conditions; the balance is excreted in the feces (Fig. 29-2). Calcium absorption can be increased to as much as 600 mg per day in the presence of **calcitriol** (the active form of vitamin D), as discussed below. To maintain calcium homeostasis, the absorption of calcium from the intestine is balanced by daily calcium losses through renal excretion (about 200 mg per day) and through secretions (primarily saliva and bile) that are eliminated in the feces (about 100 mg per day; Fig. 29-2). Compared with calcium homeostasis, phosphate homeostasis is not as highly regulated.

## REGULATION OF BONE REMODELING

Bone homeostasis can be viewed as a dynamic balance between anabolic (bone formation) and catabolic (bone resorption) processes. *Osteoblasts are the cells most responsible for anabolic activity, while **osteoclasts** are responsible for catabolic activity.* Regulation of these two cell types by hormones, mechanical factors, and cytokines determines the balance between bone formation and bone resorption (see below).

To maintain its strength over time and to respond adaptively to physical stresses, human bone is continually resorbed and reformed. This process is called **remodeling.** Because, in part, of its large surface area on which remodeling can take place, 25% of trabecular bone is remodeled each year in adults. In contrast, only 3% of cortical bone is remodeled each year. This difference is important because *pathologic situations that disturb bone remodeling preferentially affect bones with a high content of trabecular bone, such as the femoral neck and vetebral bodies.*

Remodeling is carried out by the coordinated activity of millions of cellular units—**basic multicellular units** (BMU)—consisting of osteoblasts and osteoclasts. The process of resorption begins when physical or chemical signals (discussed below) recruit osteoclasts, which begin excavating small cavities on the surface of the bone (Fig. 29-3). Osteoclasts extend villus-like projections toward the bone surface. These villi secrete proteolytic enzymes, such as collagenase, that digest the organic matrix. After attaching tightly to the bone surface, the osteoclasts create an acidic microenvironment by producing various organic acids, including lactic acid, carbonic acid, and citric acid, and by using a $H^+$-ATPase in the villi to pump protons onto the bone surface. The dissolution of hydroxyapatite can be expressed as follows:

$$(Ca)_5(PO_4)_3OH \rightarrow 5Ca^{2+} + 3PO_4^{-3} + OH^- \qquad \text{Equation 29-1}$$

The secretion of organic acids and protons by osteoclasts consumes hydroxide at the bone surface; according to Le Châtelelier's principle, $OH^-$ consumption drives the reaction to the right and results in increased dissolution of the hydroxyapatite. This is an important mechanism exploited by osteoclasts to resorb the mineral component of bone.

After a period of about 3 weeks of bone resorption, cytokines and other factors liberated from the matrix begin to stimulate osteoblast proliferation, differentiation, and activation. These osteoblasts replace the osteoclasts in the resorption cavity (lacuna), and begin to refill the cavity with concentric layers, or **lamellae,** of unmineralized organic matrix (osteoid) (Fig. 29-3). As osteoblasts continue to lay down matrix, they eventually become completely surrounded with matrix and are then called osteocytes (Fig. 29-1). As the cavity is filled with new osteoid, various factors essential for the process of mineralization are secreted by the osteoblasts. These factors include **alkaline phosphatase** and calcium-binding proteins. Alkaline phosphatase hydrolyzes various phosphate esters, including pyrophosphate, which is an inhibitor of bone mineralization. The hydrolysis of pyrophosphate also serves to increase the local concentration of inorganic phosphate. Together, the hydrolysis of pyrophosphate and liberation of

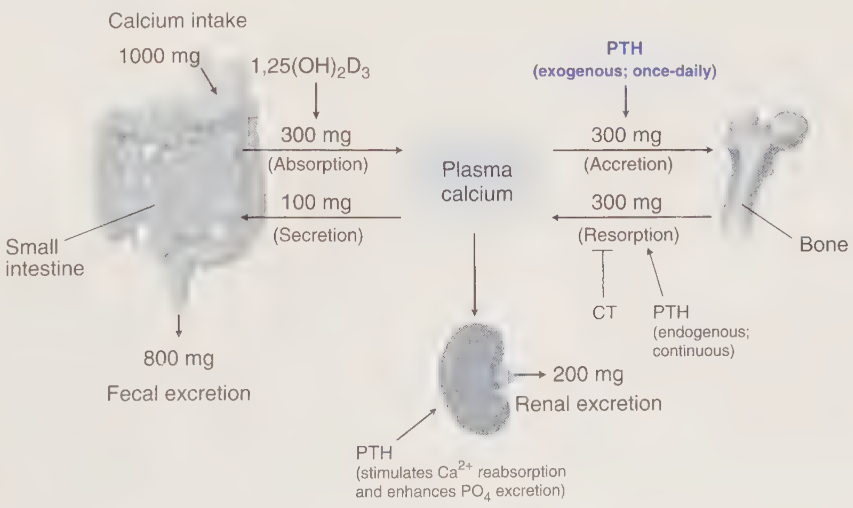


**Figure 29-2. Daily Whole-Body Calcium Balance.** In a state of whole-body calcium balance, the flux of calcium includes net uptake of 200 mg per day from the GI tract and excretion of 200 mg per day by the kidneys. Vitamin D [$1,25(OH)_2D_3$] enhances absorption of $Ca^{2+}$ from the GI tract. Endogenous secretion of parathyroid hormone (PTH) modulates bone resorption and stimulates tubular resorption of calcium in the nephron; both effects raise plasma $Ca^{2+}$. PTH also enhances urinary excretion of inorganic phosphate ($PO_4$). In contrast, exogenous once-daily injection of PTH (in blue) stimulates new bone formation (accretion). Calcitonin (CT) inhibits bone resorption.

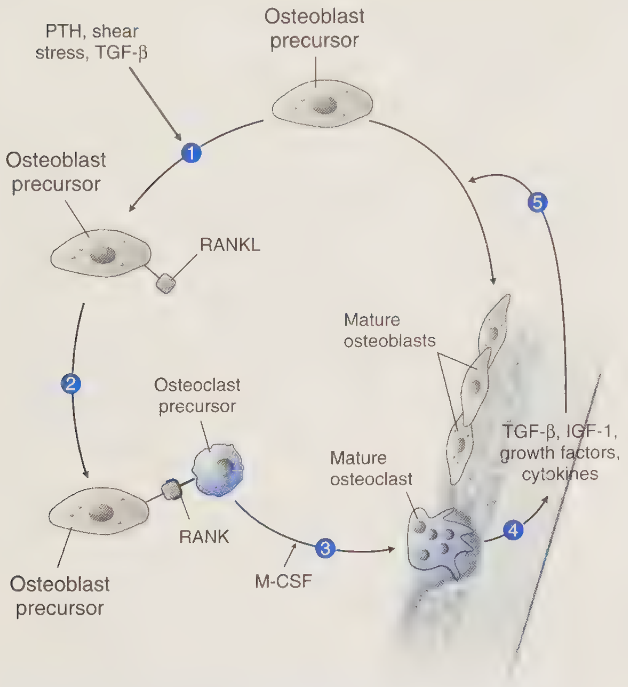


**Figure 29-3. Interaction of Osteoblasts and Osteoclasts in Bone remodeling.** Bone resorption and bone formation are coupled by the interactions between osteoblasts and osteoclasts: 1. Factors such as parathyroid hormone (PTH), shear stress, and transforming growth factor β (TGF-β) cause osteoblast precursors to express the osteoclast differentiation factor RANK-ligand (RANKL). 2. RANKL binds to RANK, a receptor expressed on osteoclast precursors. 3. The RANKL–RANK binding interaction, together with such other factors as macrophage colony stimulating factor (M-CSF), cause osteoclast precursors to differentiate into mature osteoclasts. 4. As mature osteoclasts resorb bone, such matrix-bound factors as TGF-β, insulin-like growth factor 1 (IGF-1), other growth factors, and cytokines are released. 5. These liberated factors stimulate osteoblast precursors to develop into mature osteoblasts, which begin to refill the resorption cavities excavated by the osteoclasts.

inorganic phosphate by alkaline phosphatase promote the crystallization of calcium phosphate salts. In a complementary fashion, the secretion of calcium-binding proteins increases the local concentration of calcium and, thereby, further facilitates hydroxyapatite formation.

## HORMONAL CONTROL OF CALCIUM AND PHOSPHATE

Calcium is essential for a large number of physiologic processes, such as neurotransmitter release, muscle contraction, and blood coagulation. Even small deviations in extracellular calcium levels can have serious consequences. Therefore, calcium homeostasis is very carefully regulated. Phosphate homeostasis must also be regulated, because changes in plasma phosphate concentrations affect plasma calcium levels (see below). Three main hormones—parathyroid hormone (PTH), vitamin D and, to a lesser extent, calcitonin—mediate calcium and phosphate homeostasis. In addition, glucocorticoids, thyroid hormone, and gonadal steroids have lesser effects on calcium and phosphate homeostasis. Table 29-1 summarizes the mechanisms and effects of these hormones on calcium and phosphate homeostasis.

### Parathyroid Hormone

The most important endocrine regulator of calcium homeostasis is **parathyroid hormone**, an 84-amino acid peptide hormone secreted by the parathyroid glands. The secretion of PTH is finely regulated in response to plasma calcium levels. Calcium-sensing receptors reside on the plasma membrane of chief cells in the parathyroid gland; when bound by extracellular calcium ions, these G protein-coupled receptors increase the levels of intracellular free calcium, which, in turn, decreases secretion of preformed PTH. By this mechanism, *high plasma calcium concentration suppresses PTH secretion, while low plasma calcium stimulates PTH secretion.* (Note: In many other secretory tissues, an increase in intracellular calcium enhances secretion. Thus, the parathyroid chief cell is somewhat unique in its response to changes in intracellular calcium.)

PTH acts on three organs to raise the plasma calcium concentration: It acts directly on kidney and bone, and indirectly on the GI tract (Fig. 29-4). The most rapid physiologic effects of PTH are to increase reabsorption of calcium and to decrease reabsorption of phosphate by the kidney. These actions decrease urinary excretion of calcium while increasing urinary excretion of phosphate. In this manner, PTH raises plasma calcium levels and decreases plasma phosphate concentrations. The effect of PTH on plasma phosphate concentration is important because the low solubility of calcium phosphate salts causes the plasma calcium and phosphate concentrations to be inversely related. An independent increase in plasma phosphate lowers plasma calcium; conversely, a decrease in plasma phosphate increases the concentration of free calcium. The latter condition is precisely the effect that PTH mediates via the kidney.

Another important, although slower, effect of PTH results from its direct action on bone cells. Physiologic levels of PTH stimulate cell surface PTH receptors on osteoblasts, causing these cells to increase their expression of a number of proteins. One of these PTH-induced proteins is the osteoclast differentiation factor **RANK ligand (RANKL).** RANKL binds to **RANK,** a receptor expressed on osteoclast precursor cells in the bone marrow, promoting the differentiation of these precursors into mature osteoclasts (Fig. 29-3). The ensuing increase in osteoclastic activity results in increased bone resorption and, therefore, in liberation of calcium and phosphate into the circulation. By this mechanism, the direct action of PTH on bone raises plasma calcium concentrations.

Finally, PTH raises plasma calcium by an indirect effect on the intestine. PTH stimulates the kidney not only to increase calcium reabsorption and decrease phosphate reabsorption, as described above, but also to increase the conversion of the inactive 25-hydroxy form of vitamin D to the biologically active 1,25-dihydroxy vitamin D (calcitriol). This hydroxylation takes place in cells of the proximal tubule. Calcitriol, in turn, increases calcium absorption in the small intestine (discussed below).

Although PTH has classically been considered a bone catabolic hormone, *recent evidence indicates that, under certain conditions, PTH can also increase bone formation and bone mass.* Under these conditions, it appears that PTH can enhance osteoblast survival and promote differentiation of osteoblast precursors to mature osteoblasts. Specifically, intermittent, brief (1–2 hr) interaction of PTH with its receptor

**TABLE 29-1** Summary of Endocrine Control of Calcium and Phosphate Homeostasis

| HORMONE | TARGET ORGAN | MECHANISM | NET EFFECT |
|---|---|---|---|
| PTH | GI tract | ↑ $Ca^{2+}$ absorption via vitamin D action | ↑ $[Ca^{2+}]$ ↓$[P_i]$ |
| | Kidney | ↑ $Ca^{2+}$ reabsorption; ↓ $P_i$ reabsorption | |
| | Bone | ↑ Osteoclast activity (continuous PTH) | ↑ $[Ca^{2+}]$ ↓$[P_i]$; ↓ Bone mass |
| | | ↑ Osteoblast activity (once-daily PTH) | ↑ Bone mass |
| Vitamin D | GI tract | ↑ $Ca^{2+}$ and $P_i$ absorption | ↑ $[Ca^{2+}]$ ↑$[P_i]$ |
| | Kidney | ↑ $Ca^{2+}$ and $P_i$ reabsorption | |
| | Bone | ↑ Osteoclast number and activity | |
| | Parathyroid gland | Inhibition of PTH synthesis | |
| Calcitonin | Bone | ↓ Osteoclast activity | ↓ $[Ca^{2+}]$ ↓$[P_i]$ |
| Glucocorticoids | GI tract | ↓ $Ca^{2+}$ absorption, leading to ↑ PTH | |
| | Kidney | ↓ $Ca^{2+}$ and $P_i$ reabsorption | Osteopenia |
| | Bone | ↓ Osteoblast activity; ↑ osteoblast apoptosis | Osteoporosis |
| Thyroid hormone | Bone | Bone resorption > formation | Osteopenia |
| Gonadal steroids | Bone | ↓ Osteoclast activity; ↑ osteoclast apoptosis<br>↓ Osteoblast apoptosis | ↓ Bone resorption |

$P_i$, inorganic phosphate.

on mature osteoblasts stimulates $G\alpha_s$, which increases adenylyl cyclase activity, which in turn increases intracellular cAMP. The PTH-induced increase in cAMP has an anti-apoptotic effect on the osteoblasts. In addition, the increase in cAMP promotes osteoblast release of IGF-1, which induces osteoblast precursor cells in the bone marrow to differentiate into mature osteoblasts (Fig. 29-3). Although intermittent PTH also induces the production of such cytokines as IL-6 by stromal precursor cells, and these cytokines ultimately stimulate osteoclast proliferation, this catabolic effect is dominated at first by the PTH-induced activation of osteoblasts.

In contrast, continuous interaction of PTH with its receptor on mature osteoblasts stimulates these cells to produce IGF-binding proteins (IGFBP). The resulting decrease in IGF-1 levels reduces the differentiation of osteoblast precursor cells. In addition, as described above, continuous PTH induces the expression of such osteoclast differentiation factors as RANKL on stromal cells and osteoblasts. The net result of continuous PTH exposure is that osteoclasts outnumber osteoblasts, and bone resorption overrides bone formation. Conceptually, the differential effects of intermittent versus continuous PTH exposure are similar to the situation with GnRH: Recall that pulsatile GnRH stimulates LH and FSH secretion by the anterior pituitary gland; whereas, continuous GnRH suppresses release of these gonadotropins (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). Because of this differential effect of PTH, intermittent administration of PTH by once-daily injection or by some other drug delivery technology could allow the use of PTH to promote osteoblast activity and, thereby, to increase bone matrix production, bone mineral density, bone quality, and bone mass (see below).

## Vitamin D

Despite its name, **vitamin D** is produced by the body in sufficient amounts that it is not required in the diet under normal conditions. Because it is produced endogenously and because it travels in the blood to effect responses in distant target tissues, vitamin D is properly considered a hormone. The term vitamin D applies to two related compounds, **cholecalciferol** and **ergocalciferol.** Cholecalciferol, or vitamin $D_3$, is the form synthesized in the skin from a precursor of cholesterol called 7-dehydrocholesterol. This biosynthetic pathway is stimulated by exposure of the skin to the ultraviolet radiation in sunlight (Fig. 29-5). Ergocalciferol, or vitamin $D_2$, is the form produced by plants. (Vitamin $D_2$ is also the form present in many commercial preparations, as well as the form added to milk.) Vitamin $D_2$ and $D_3$ have equal biological activity, and the "D" in compounds discussed below refers to both the $D_2$ and the $D_3$ forms of the hormone.

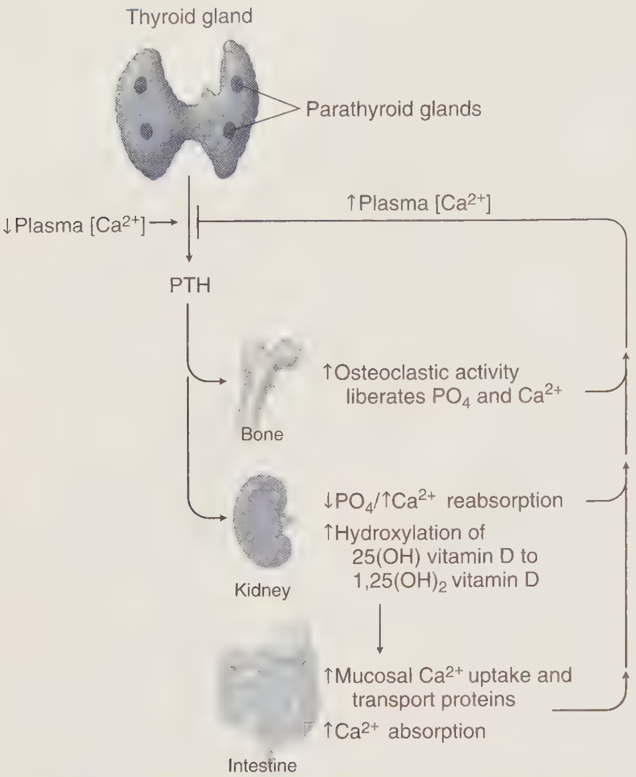

Thyroid gland
Parathyroid glands
↓Plasma [Ca²⁺]
↑Plasma [Ca²⁺]
PTH
↑Osteoclastic activity liberates PO₄ and Ca²⁺
Bone
↓PO₄/↑Ca²⁺ reabsorption
↑Hydroxylation of 25(OH) vitamin D to 1,25(OH)₂ vitamin D
Kidney
↑Mucosal Ca²⁺ uptake and transport proteins
↑Ca²⁺ absorption
Intestine

**Figure 29-4. Summary of the Actions of PTH on Bone, Kidney, and Intestine.** Decreased plasma [$Ca^{2+}$] is the primary stimulus for parathyroid hormone (PTH) secretion by the parathyroid glands. PTH acts to raise plasma $Ca^{2+}$ levels via its effects on bone, kidney, and intestine. In bone, PTH promotes increased differentiation of osteoclast precursors into mature osteoclasts. Osteoclasts resorb bone and, thereby, liberate inorganic phosphate ($PO_4$) and $Ca^{2+}$ into the plasma. In the kidney, PTH increases the tubular reabsorption of $Ca^{2+}$ and decreases the distal tubular reabsorption of $PO_4$. In addition, PTH stimulates proximal tubule cells to hydroxylate 25(OH) vitamin D, forming $1,25(OH)_2$ vitamin D. $1,25(OH)_2$ vitamin D then stimulates intestinal absorption of $Ca^{2+}$ by increasing the expression of mucosal $Ca^{2+}$ uptake and transport proteins. Note that the effect of PTH on the intestine is indirect, via increased renal synthesis of the active form of vitamin D. In a tightly controlled negative feedback loop, increased plasma [$Ca^{2+}$] inhibits further PTH secretion by the parathyroid glands.

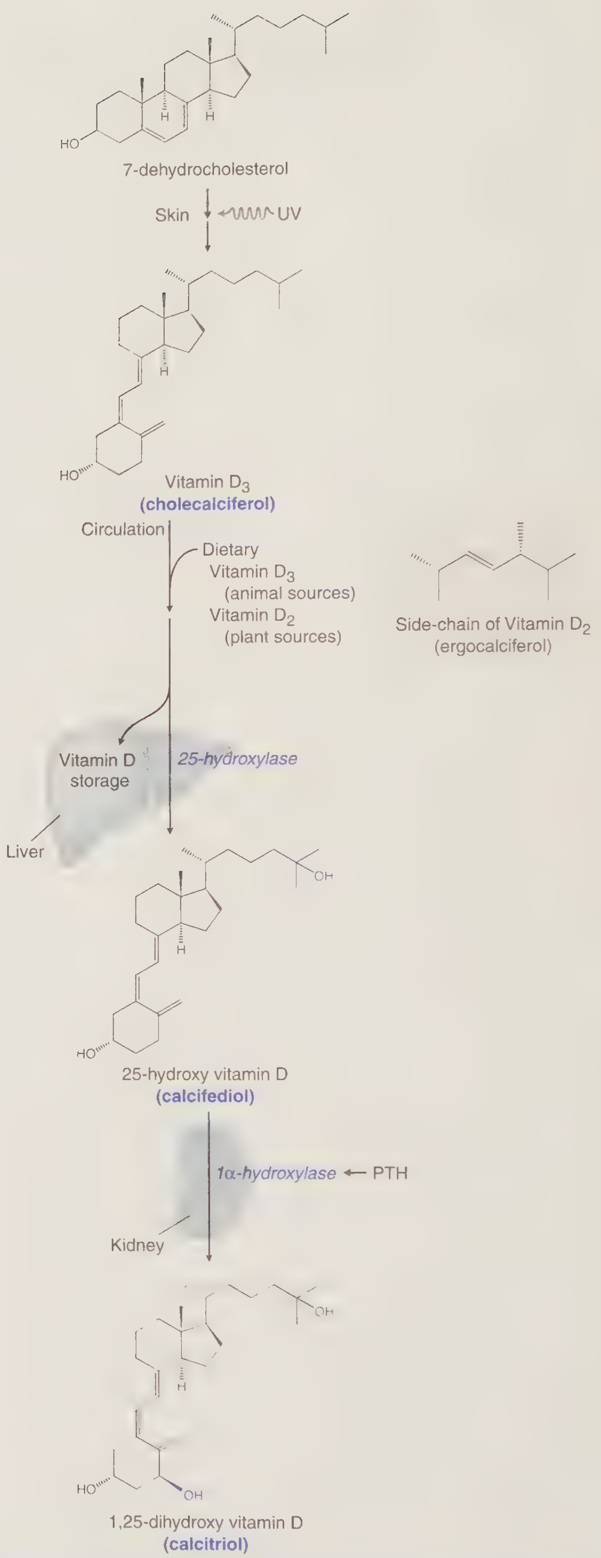

HO
H
H
7-dehydrocholesterol
Skin
UV
H
HO
Vitamin D₃
(cholecalciferol)
Circulation
Dietary
Vitamin D₃
(animal sources)
Vitamin D₂
(plant sources)
Side-chain of Vitamin D₂
(ergocalciferol)
Vitamin D storage
25-hydroxylase
Liver
OH
H
HO
25-hydroxy vitamin D
(calcifediol)
1α-hydroxylase ← PTH
Kidney
OH
H
HO
OH
1,25-dihydroxy vitamin D
(calcitriol)

**Figure 29-5. Photobiosynthesis and Activation of Vitamin D.** Both endogenous and exogenous vitamin D are converted to 25-hydroxy vitamin D in the liver and then to calcitriol in the kidney. Calcitriol is the active metabolite of vitamin D. Endogenous vitamin $D_3$ is synthesized in the skin from 7-dehydrocholesterol, in a reaction that is catalyzed by ultraviolet light. Exogenous vitamin D can be provided as $D_3$ (from animal sources) or as $D_2$ (from plant sources); $D_3$ and $D_2$ have the same biological activity. Parathyroid hormone (PTH) increases the activity of 1α-hydroxylase in the kidney and, thereby, stimulates the conversion of 25-hydroxy vitamin D to calcitriol.

Whether from an endogenous (skin) or an exogenous (dietary) source, vitamin D travels to the liver, where it is either stored or converted to calcifediol [25-hydroxy vitamin D, or 25(OH)D] by the first of two enzymatic hydroxylation steps. The second enzymatic hydroxylation, which is PTH-dependent and takes place in the proximal tubule of the kidney, converts calcifediol to the final, active form of vitamin D called calcitriol [1α,25-hydroxy vitamin D, or $1,25(OH)_2D_3$].

*Calcitriol's primary effect on calcium balance is in the small intestine, where it increases the absorption of dietary calcium.* Calcitriol enhances $Ca^{2+}$ absorption by acting on nuclear receptors in the enterocyte to up-regulate the expression of genes coding for a number of brush border proteins. Calcitriol also promotes the transcellular transport of $Ca^{2+}$ through the enterocyte by inducing the expression of: (1) a calcium uptake pump on the luminal surface of the enterocyte; (2) calbindin, an intracellular $Ca^{2+}$-binding protein; and (3) an ATP-dependent $Ca^{2+}$ pump that extrudes $Ca^{2+}$ from the enterocyte into the surrounding capillaries.

Calcitriol has less well understood effects on other target organs, including the parathyroid gland, bone, kidneys, and the immune system. Calcitriol binds to nuclear receptors in parathyroid cells and, thereby, inhibits PTH synthesis and release. In bone, calcitriol increases osteoclast number and activity, resulting in increased bone resorption. Some recent evidence suggests that calcitriol (or analogues of calcitriol) may also increase bone formation. In the distal tubule of the kidney, calcitriol increases the reabsorption of both calcium and phosphate. In the immune system, calcitriol production by macrophages may act as a local suppressant of adaptive immune cells; this observation has led to the use of vitamin D in the treatment of psoriasis, an autoimmune dermatologic disease.

### Calcitonin

The third major hormone involved in calcium homeostasis is **calcitonin.** Calcitonin is a 32-amino acid peptide that is produced and released by parafollicular C cells of the thyroid gland in response to hypercalcemia. *Calcitonin binds directly to receptors on osteoclasts; this binding inhibits the resorptive activity of the osteoclasts and, thereby, decreases plasma calcium levels.* In adult humans, there is only a weak effect of endogenous calcitonin on plasma calcium levels, and the elimination of calcitonin secretion after thyroidectomy generally causes no significant changes in plasma calcium levels. Nevertheless, exogenous calcitonin does have a therapeutic application in the treatment of osteoporosis, as discussed below.

### Glucocorticoids, Thyroid Hormone, and Gonadal Steroids

Although PTH, vitamin D and, to a lesser extent, calcitonin are the primary regulators of calcium homeostasis, several other endogenous hormones have important effects on bone mineral metabolism. These hormones include glucocorticoids, thyroid hormone, estrogens, and androgens.

Pharmacologic doses of glucocorticoids decrease both intestinal absorption of calcium and renal tubular reabsorption of calcium. Although both of these effects would tend to lower plasma calcium levels, glucocorticoid use is not associated with hypocalcemia. In part, this may be explained by an increase in PTH that is stimulated by the decrease in intestinal calcium absorption. In addition, glucocorticoids have a direct effect on bone by inhibiting osteoblast maturation and osteoblast activity, and by promoting osteoblast apoptosis. Thus, glucocorticoids preserve plasma calcium within normal limits, but at the expense of bone integrity. In fact, prolonged administration of pharmacologic doses of glucocorticoids causes osteoporosis. It is important, when taking the history of a patient such as MS, to determine whether she has ever taken glucocorticoids for an extended period of time, because this could represent a significant risk factor for osteoporosis.

Excess thyroid hormone also increases bone turnover. By stimulating bone resorption more than bone formation, prolonged high levels of thyroid hormone can cause osteopenia. In fact, osteopenia can be a manifestation of hyperthyroidism. The evaluation of MS's osteoporosis should, therefore, include a determination of her TSH level to rule out hyperthyroidism (see Chapter 25).

Estrogens and androgens exert indirect inhibitory effects on osteoclasts and, thereby, slow the rate of bone turnover and bone loss. Specifically, the gonadal steroids inhibit the production by osteoblasts of cytokines—such as interleukin-6—that recruit and activate osteoclasts. Estrogen also has a pro-apoptotic effect on the osteoclasts themselves and an anti-apoptotic effect on osteoblasts and osteocytes. As described in more detail in Chapter 27, Pharmacology of Reproduction estrogen exerts its actions principally by binding to the estrogen receptor (ER), which is a nuclear transcription factor. Binding of estrogen facilitates dimerization of the ER, allowing the estrogen–ER complex to recruit co-activator or co-repressor molecules and bind to promoter regions of target genes. In this way, estrogen regulates the transcription of target genes encoding, for example, the cytokines that are important in bone turnover.

## PATHOPHYSIOLOGY

Mineral homeostasis is crucial for the integrity of bone. Mineral imbalances that result in too little calcium and phosphate lead to such bone diseases as **rickets** and **osteomalacia.** Excess secretion of parathyroid hormone, or decreased responsiveness to signals from the calcium-sensing receptor, can cause hypercalcemia. Conversely, decreased action of parathyroid hormone can lead to hypocalcemia. Bone turnover, including repeated cycles of bone resorption and bone formation, is required to maintain the integrity of the skeleton. When this cycling is disrupted, pathophysiologic states can result in which bone resorption exceeds bone formation, either globally (**osteoporosis**) or locally (**Paget's disease**). Finally, the pathophysiology of chronic renal failure involves a complex interplay between decreased mineral absorption and **secondary hyperparathyroidism.** A summary of the various diseases of bone mineral homeostasis, including their mechanisms, clinical features, and treatments, is provided in Table 29-2.

## RICKETS AND OSTEOMALACIA

*Rickets and osteomalacia refer to deficient bone mineralization caused by vitamin D deficiency. Rickets is a disorder of growing bone, affecting both the growing bone and the epiphyseal growth plate in children. Osteomalacia is a disorder of bone that has ceased to grow, causing undermineralization of the bone matrix in adults.* Inadequate levels of vitamin D lead to inadequate intestinal absorption of calcium and phosphate. Low plasma calcium induces PTH secretion, which raises plasma calcium through increased bone resorption while keeping plasma phosphate level low through increased renal phosphate excretion. Both the increase in PTH and the low plasma phosphate levels contribute to defective bone mineralization. Rickets presents as a severe condition with kyphosis, lordosis, and bowing of the limbs. Skeletal deformity rarely occurs in osteomalacia, but undermineralization in this condition weakens the bone, increasing the incidence of fractures. Severe osteomalacia can be difficult to distinguish from osteoporosis. *Osteoporosis, unlike osteomalacia, is associated with reduced production of osteoid as well as undermineralization of bone.*

TABLE 29-2 Mechanisms, Clinical Features, and Treatments for Common Bone Diseases

| DISEASE | MECHANISM | CLINICAL FEATURES | TREATMENT |
|---|---|---|---|
| Rickets | | Skeletal deformities in children<br>Osteomalacia in adults | |
| Nutritional | Inadequate sunlight or dietary ↓ vit D | | Vitamin D |
| Vitamin D resistant | Defect in renal reabsorption of phosphate and production of vit D | | Oral phosphate<br>Calcitriol |
| Type I vit D dependent | ↓ Production of $1,25(OH)_2D$ | | Oral phosphate<br>Calcitriol |
| Type II vit D dependent | Defective receptors for $1,25(OH)_2D$ | | Calcitriol (high dose) |
| Primary hyperparathyroidism | ↑ PTH → ↑ bone resorption → hypercalcemia | Osteoporosis<br>Nephrolithiasis<br>Osteitis fibrosa cystica<br>Depression | Surgical removal<br>Calciomimetic agents (investigational) |
| Familial hypocalciuric hypercalcemia (FHH) | Mutation in $Ca^{2+}$-sensing receptor | Hypocalciuria<br>Hypercalcemia<br>Hypermagnesemia | None |
| Hypoparathyroidism | Decreased activity or absence of parathyroid gland; → hypocalcemia and hyperphosphatemia | Neuromuscular excitability<br>Tetany<br>Depression | Calcium<br>Vitamin D |
| Pseudohypoparathyroidism | Impaired response to PTH → hypocalcemia | Short stature<br>Short metacarpals | Calcium<br>Vitamin D |
| Osteoporosis<br>Senile<br>Postmenopausal | Bone resorption > bone formation<br>↓ Estrogen | Fragile bone<br>Vulnerable to fracture | Calcium<br>Vitamin D<br>Bisphosphonates<br>Raloxifene (SERM)<br>Calcitonin<br>PTH |
| Paget's disease | ↑ Local bone turnover | Bone pain<br>Hearing loss<br>High-output cardiac failure | Bisphosphonates<br>Calcitonin |
| Chronic renal failure | ↓ Excretion of phosphate<br>↓ Production of $1,25(OH)_2D$ | Osteomalacia<br>Osteitis fibrosa cystica | Phosphate restriction<br>Activated vitamin D |

Rickets can be divided into several subtypes based on its pathophysiology. The nomenclature is confusing, but all the disorders involve a disruption in vitamin D homeostasis.

**Nutritional rickets** results from inadequate exposure to sunlight or dietary deficiency of vitamin D. Treatment with vitamin D corrects this condition.

**Hypophosphatemic vitamin D-resistant rickets** is an X-linked genetic disorder characterized by a low plasma phosphate level and a $1,25(OH)_2D$ level that is inappropriately low for the degree of hypophosphatemia. Because phosphate is crucial to bone mineralization, persistently low phosphate levels cause the clinical manifestations of rickets. A defect in renal reabsorption of phosphate seems to be the underlying cause in most cases; whereas, a defect in the renal production of $1,25(OH)_2D$ has also been observed. This condition is termed "vitamin D-resistant" because children affected with this type of rickets are refractory to the usual replacement dose of vitamin D. High pharmacologic doses of calcitriol, however, do result in clinical improvement.

**Vitamin D-dependent rickets** is divided into two subtypes. Type I is an autosomal recessive disorder caused by a deficiency in the production of $1,25(OH)_2D$. Type II is an autosomal recessive disorder caused by one of several mutations in the $1,25(OH)_2D$ receptor that lead to abnormalities in the target tissue responses to $1,25(OH)_2D$. Patients with type I vitamin D-dependent rickets can be treated with replacement doses of calcitriol; whereas, patients with type II either do not respond to treatment with calcitriol (if their $1,25(OH)_2D$ receptor is missing) or require high pharmacologic doses of calcitriol (if their $1,25(OH)_2D$ receptor is mutated).

## HYPERPARATHYROIDISM

The various etiologies of hyperparathyroidism can be divided into **primary hyperparathyroidism,** secondary hyperparathyroidism, and decreased responsiveness of parathyroid cells to the calcium-sensing receptor.

A **parathyroid adenoma** or **parathyroid hyperplasia** underlies 95% of the cases of primary hyperparathyroidism. Both adenoma and hyperplasia cause persistent and excessive secretion of PTH that, in turn, can lead to hypercalcemia and the development of calcium-containing renal calculi (stones). The constellation of symptoms in severe primary hyperparathyroidism is said to include "painful bones, renal stones, abdominal groans, and psychic moans." Prolonged elevation of circulating PTH favors bone resorption over bone formation, causing osteoporosis as well as **osteitis fibrosa cystica,** a condition marked by bone pain and by the replacement of areas of bone with fibrous connective tissue. Hypercalcemia decreases neuromuscular excitability which leads to fatigue, constipation, and weakness, and causes psychiatric disorders including depression and mental confusion. Current treatment for primary hyperparathyroidism consists of surgical parathyroidectomy, although calciomimetic agents (which act on the calcium receptor on parathyroid cells to inhibit parathyroid hormone secretion) are currently under development.

Secondary hyperparathyroidism often follows a chronic reduction in plasma calcium that leads to oversecretion of PTH by the parathyroid glands. The underlying cause is typically **renal failure,** which increases plasma phosphate levels and decreases $1,25(OH)_2D$ production (see the discussion of chronic renal failure below for more detail). Although renal failure and its associated symptoms dominate the clinical picture of secondary hyperparathyroidism, the associated skeletal abnormalities can also be highly debilitating.

**Familial hypocalciuric hypercalcemia** (FHH) is an autosomal dominant disorder with a benign presentation. It is marked by a fractional calcium excretion of less than 1%. In this disorder, the kidney reabsorbs more than 99% of filtered calcium, even in the presence of hypercalcemia. Mutations in the calcium-sensing receptor on the surface of parathyroid and renal cells underlie the pathophysiology of this disorder. Some mutations render the receptor less responsive to calcium, so that a higher concentration of calcium is required to inhibit PTH secretion. Persistent PTH secretion stimulates renal absorption of calcium as well as magnesium, leading to hypercalcemia, hypocalciuria, and hypermagnesemia. In other cases, mutations cause reduced expression of the calcium-sensing receptor, leading to the same clinical presentation. FHH is a benign disease; patients present with no symptoms but do have signs associated with mild hypercalcemia. Presently, there is no definitive treatment for FHH because subtotal parathyroidectomy does not cure the disorder.

## HYPOPARATHYROIDISM

Hypoparathyroidism results from a deficiency in the secretion of PTH attributable to any of several causes, including inadvertent surgical injury or removal of the parathyroid glands, congenital absence of the glands, or autoimmune destruction of the glands. Low plasma levels of PTH lead to hypocalcemia and hyperphosphatemia. The major symptoms of hypoparathyroidism are associated with hypocalcemia. Neuromuscular excitability results from the fact that low plasma calcium lowers the threshold for firing of action potentials. Severe hypocalcemia can lead to tetany, including life-threatening laryngospasm and even convulsions. Hypocalcemia also causes mental status changes such as anxiety, depression, and hallucinations. Bone mineralization is typically not reduced, in part because of the high plasma phosphate level. Treatment is aimed at raising plasma calcium levels with oral calcium and vitamin D supplements.

**Pseudohypoparathyroidism** comprises a complex group of genetic disorders that can mimic hypoparathyroidism, except that, in these cases, hypocalcemia and hyperphosphatemia are associated with normal or increased plasma levels of PTH. Hypocalcemia is present despite the normal to high level of plasma PTH because of impaired target tissue responsiveness to PTH, often caused by mutations in the guanine nucleotide binding protein $G_s$. As noted above, the PTH receptor is a G protein-coupled receptor that signals via $G_s$ (and, to a lesser extent, via $G_q$). When secreted PTH does not induce the kidney to reabsorb calcium or the bone to release calcium, then low plasma calcium levels persist, leading to further secretion of PTH and to symptoms of hypocalcemia. Because downstream effects of PTH receptor signaling are also essential for bone morphogenesis, patients with pseudohypoparathyroidism often present early in life with developmental defects. Although there is no treatment for the developmental abnormalities of pseudohypoparathyroidism, plasma calcium can be maintained at normal levels with oral calcium and vitamin D supplements.

## OSTEOPOROSIS

Osteoporosis is a common condition that results from decreased production of bone matrix and decreased bone mineral density (BMD). The reduced bone mass predisposes to fracture upon minimal trauma. Normal BMD is defined as a value that is within one standard deviation of the mean BMD in young adults (the "T score"), as determined by radiographic techniques. Values of 1.0 to 2.5 standard deviations below the mean are defined as osteopenia, and values more than 2.5 standard deviations below the mean are defined as osteoporosis.

Osteoporosis leads to an increased risk of fracture, especially in the vertebrae, femoral neck, and radius (spine, hip, and Colles' fractures, respectively). MS's presentation, with back pain from a compression fracture in a lumbar vertebra, is typical.

There are two categories of osteoporosis—primary and secondary. There are two types of primary osteoporosis—**senile** and **postmenopausal osteoporosis.** Peak bone mass, which is achieved in young adulthood, is determined by several factors, including such genetic factors as the alleles for the vitamin D receptor, dietary calcium levels, hormonal state, and physical activity. *Once peak bone mass is attained, there is a gradual decline in bone mass during mid to late adult life.* This decline probably results from imperfections in the bone remodeling process; osteoblast-mediated bone formation does not completely keep pace with osteoclast-mediated bone resorption. Moreover, with age, the osteoblasts have a reduced capacity to proliferate, to synthesize organic bone matrix, and to respond to growth factors. As a result, there is, on average, a 0.7% loss of bone mass per year. This rate of bone loss can, eventually, lead to senile osteoporosis in old age (Fig. 29-6).

Postmenopausal osteoporosis begins in the first 3–5 years after menopause, when there is a rapid loss in bone mass related to the marked decline in estrogen production. MS, for example, was diagnosed with osteoporosis about 6 years after completing menopause. The decline in estrogen levels leads to an increase in osteoclast activity and bone turnover rate, which causes an imbalance between bone formation and bone re-

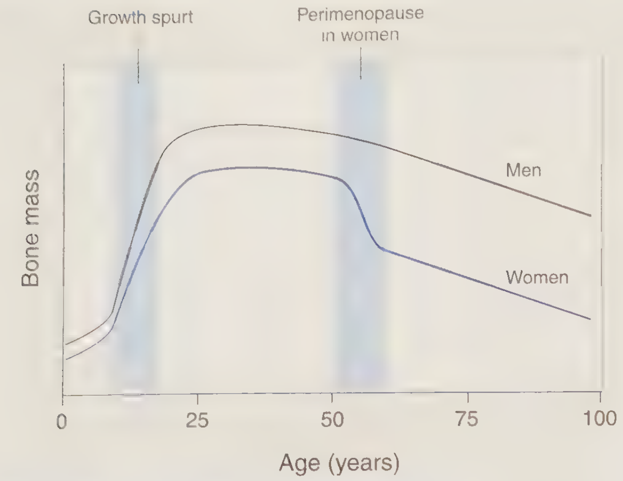


**Figure 29-6. Bone Mass as a Function of Age.** In both men and women, bone mass increases with age until a peak is reached in young adulthood, after which bone mass gradually declines by approximately 0.7% per year. In women, the onset of menopause precipitates a sharp decline in bone mass as the decrease in estrogen production leads to increased bone resorption. As bone mass decreases with age, the skeleton may become sufficiently fragile that minor trauma can cause fractures. The goal of antiresorptive agents is to decrease the rate of decline of bone mass. In contrast, bone anabolic agents can be used to increase bone mass and, thereby, correct situations in which significant loss of bone mass has already occurred.

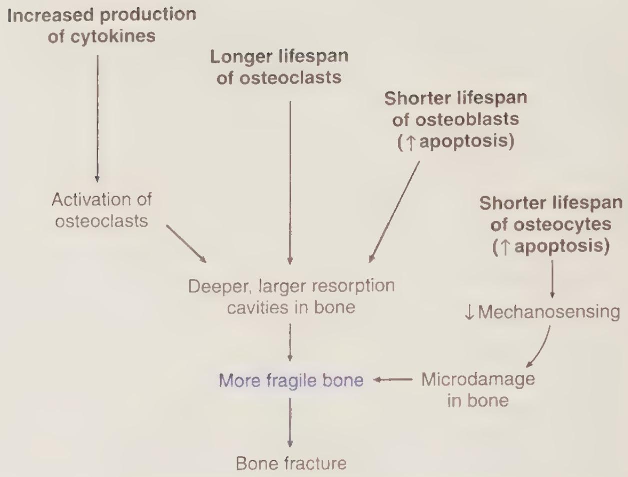

Increased production of cytokines
Longer lifespan of osteoclasts
Shorter lifespan of osteoblasts (↑ apoptosis)
Activation of osteoclasts
Shorter lifespan of osteocytes (↑ apoptosis)
Deeper, larger resorption cavities in bone
↓ Mechanosensing
More fragile bone
Microdamage in bone
Bone fracture

**Figure 29-7. Pathophysiologic Basis of Osteoporosis.** Several interrelated factors contribute to the development of osteoporosis. Many of these factors are activated by the decline in estrogen levels in perimenopausal women. Disinhibited production of cytokines and other regulatory molecules leads to the activation of osteoclasts. Decreased estrogen allows these osteoclasts to have a longer functional lifespan; conversely, the lack of estrogen promotes apoptosis in osteoblasts and osteocytes. The resulting imbalance between osteoclast and osteoblast activity leads to the formation of deep and large resorption cavities, which make the bone fragile and prone to fracture. The relative paucity of osteocytes impairs the mechanosensory network on which repair of microdamage in bone depends. Increased microdamage also predisposes to bone fragility and eventual fracture.

sorption. The longer lifespan of osteoclasts in the absence of estrogen allows these cells to excavate deeper cavities in trabecular bone, leading to bone remodeling characterized by widely spaced and thin trabeculae with fewer interconnections. These remodeled trabeculae are structurally weaker in weight-bearing regions than the well-connected, closely spaced, thick trabeculae characteristic of bone in premenopausal women. In the cortical bone, deeper cavities coalesce to form porous spaces. The lack of estrogen also leads to increased apoptosis of osteoblasts, rendering these cells unable to keep pace with the osteoclasts, and to increased apoptosis of osteocytes, impairing the mechanosensory network that detects microdamage and stimulates bone repair. Increased bone resorption and the accumulation of microdamage lead to increased bone fragility. In summary, *the fragile bone in postmenopausal osteoporosis results from a bone turnover defect characterized by increased osteoclast and decreased osteoblast activity, deeper and larger resorption cavities, and impairment of the osteocyte mechanosensory network* (Fig. 29-7).

Axial bones are more prone to osteoporosis than appendicular bones. As discussed above, remodeling takes place to a greater degree in trabecular bone than in compact bone, and appendicular bones, such as the long bones of the limbs, contain considerable amounts of compact cortical bone, while axial bones, such as the spine and pelvis, contain predominantly trabecular bone. Within 25 to 35 years after menopause, women may lose as much as 35% of their cortical bone mass and as much as 50% of their trabecular bone mass.

Certain systemic illnesses and medications induce **secondary osteoporosis.** Common causes include thyrotoxicosis, hyperparathyroidism, high doses of glucocorticoids, and alcohol abuse. Secondary osteoporosis is best treated through resolution of the underlying cause.

## PAGET'S DISEASE

Paget's disease is caused by localized increases in bone turnover. The etiology of the disease is uncertain, although current evidence hypothesizes that a **paramyxovirus** may be the causative agent. This virus may induce infected cells to produce IL-6, which can stimulate osteoclast proliferation and activation. The progression of Paget's disease is generally divided into three stages: an initial stage of local osteoclastic bone resorption, followed by a stage of increased osteoblast activity, and eventually the end stage characterized by areas of disorganized bone that are not structurally stable, leading to deformity. Pain, a common symptom of Paget's disease, is caused by a combination of microfractures and bone overgrowth that can compress spinal and cranial nerve roots. When ossicles in the ears or the auditory nerve foramina are affected, patients may present with hearing loss. Bones affected by Paget's disease can develop arteriovenous shunts that can increase preload and lead to high-output cardiac failure. Paget's disease is also a risk factor for the development of osteosarcoma. Bisphosphonates (see below) are commonly used to treat Paget's disease because these drugs can significantly slow bone turnover rates.

## CHRONIC RENAL FAILURE

Chronic renal failure affects bone mineral homeostasis mainly through secondary hyperparathyroidism, which enhances the resorption of bone. Under the influence of secondary hyperparathyroidism, bones develop both osteomalacia (decreased mineralization) and osteitis fibrosa cystica (increased osteoclastic resorption of bone with replacement by fibrous tissue). *Hyperparathyroidism in chronic renal failure stems from the interplay of several factors, including decreased production of 1,25(OH)$_2$D, hypocalcemia, and hyperphosphatemia* (Φιγ. 29–8). Each of these factors originates as a result of a decrease in renal function, manifested as an impairment in both renal synthetic ability (important for, among other processes, the $1_a$ hydroxylation step in 1,25(OH)$_2$D synthesis) and renal tubular function (important for phosphate excretion).

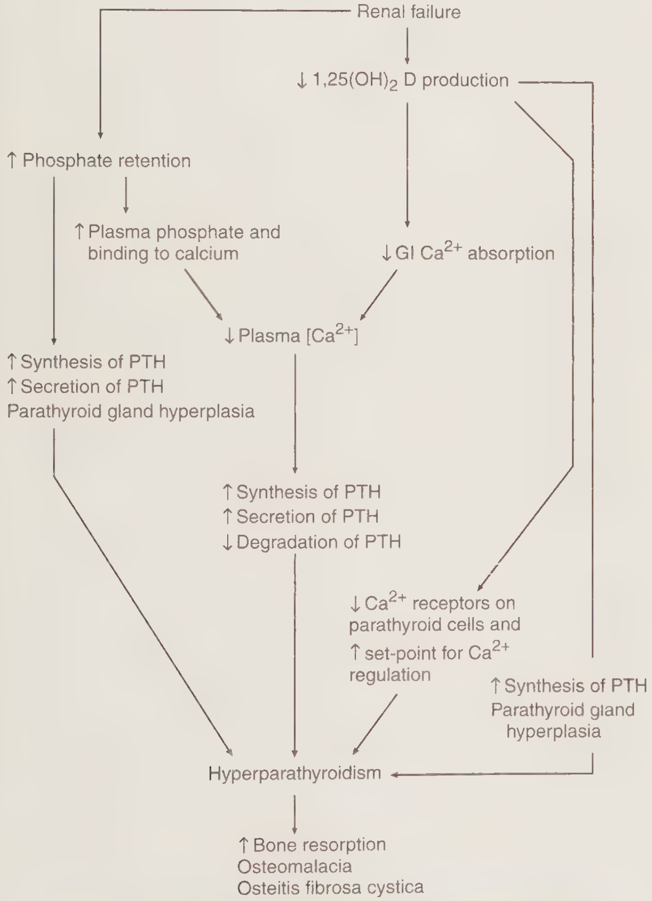


**Figure 29-8. Pathophysiologic Basis for Osteomalacia and Osteitis Fibrosa Cystica in Chronic Renal Failure.** In chronic renal failure, compromised renal function leads to decreased 1,25(OH)$_2$ vitamin D synthesis and decreased phosphate excretion. The decrease in 1,25(OH)$_2$ vitamin D causes decreased gastrointestinal (GI) absorption of $Ca^{2+}$, while the increased phosphate retention causes an increase in the levels of plasma phosphate, which complexes with $Ca^{2+}$. Therefore, by these two mechanisms, chronic renal failure leads to hypocalcemia. Hypocalcemia stimulates synthesis and secretion of parathyroid hormone (PTH) and suppresses degradation of PTH. Decreased levels of 1,25(OH)$_2$ vitamin D stimulate PTH synthesis and parathyroid gland hyperplasia, and lead to a decreased number of $Ca^{2+}$ receptors on parathyroid cells and an elevated set-point for $Ca^{2+}$regulation. Hyperphosphatemia may also stimulate increased synthesis and secretion of PTH directly. This combination of complex regulatory events leads to hyperparathyroidism, a syndrome characterized by increased bone resorption, osteomalacia, and osteitis fibrosa cystica.

Inadequate levels of 1,25(OH)$_2$D lead to inadequate intestinal absorption of calcium. The resulting hypocalcemia stimulates PTH synthesis and secretion, and suppresses PTH degradation. The low levels of 1,25(OH)$_2$D are also thought to cause a reduction in calcium receptor synthesis in parathyroid cells. The decrease in the number of calcium receptors on the plasma membranes of parathyroid cells raises the set-point for calcium regulation, so that a higher concentration of calcium is required to suppress PTH secretion. By this mechanism, hyperparathyroidism can persist even in the setting of hypercalcemia. In addition, recent evidence suggests that 1,25(OH)$_2$D normally suppresses both growth of the parathyroid gland and transcription of the PTH gene. Therefore, the deficiency of 1,25(OH)$_2$D in chronic renal failure could exacerbate secondary hyperparathyroidism by several different mechanisms. This understanding has led to the development of vitamin D analogues as potential treatments for the metabolic sequelae of chronic renal failure (see below).

Hyperphosphatemia, resulting from decreased renal excretion of phosphate, further exacerbates the hypocalcemia of chronic renal failure. Hyperphosphatemia induces hypocalcemia by altering the equilibrium for hydroxyapatite formation and dissolution, as described in Equation 29-1. Extravascular precipitates of calcium phosphate can further damage other tissues.

## PHARMACOLOGIC CLASSES AND AGENTS

Significant advances have occurred in recent years in the treatment of osteoporosis and other bone disorders. The relevant pharmacologic agents can be divided into two main categories: *drugs that inhibit bone resorption* and *drugs that stimulate bone formation.* Most drugs used to treat disorders of bone homeostasis are antiresorptive agents, which act by suppressing osteoclastic bone resorption. Because bone resorption and bone formation are closely coupled processes, a decrease in one typically leads to a decrease in the other. As a result, antiresorptive drugs induce little net gain in bone mass, although they largely prevent progressive loss of skeletal mass. The modest increase in bone mineral density during the first year or two of antiresorptive therapy is thought to represent compensatory mineralization of resorption cavities that had been produced during the previous period of excessive bone resorption. In contrast, anabolic agents, which stimulate bone formation, promise to increase bone mass (both matrix and mineral) significantly.

## ANTIRESORPTIVE AGENTS

### Hormone Replacement Therapy (HRT)

Estrogen has been one of the most commonly prescribed drugs for postmenopausal osteoporosis. Estrogen acts to reduce bone resorption by suppressing the transcription of cytokines such as IL-6 that induce osteoclast proliferation, differentiation, and activation. Estrogen also promotes osteoclast apoptosis, while increasing the lifespans of osteoblasts and osteocytes. Estrogen does not increase bone formation, but it does maintain bone mass or slow bone loss through its antiresorptive properties.

Estrogen is administered cyclically with a progestational agent to reduce the risk of endometrial cancer (see Chapter 27). HRT also relieves postmenopausal symptoms including hot flashes and vaginal dryness. Compliance is often a problem with HRT, because the side effects of estrogen, including reinitiation of uterine bleeding, breast pain, and breast tenderness, can cause patients to discontinue treatment. HRT also increases the risk of venous thromboembolism, because estrogen promotes the hepatic synthesis of clotting factors. For many women, the greatest concern regarding HRT is the increased long-term risk of breast cancer, which is small but statistically significant. In 2002, a large government (National Institutes of Health) sponsored study concluded that the increased risks of cardiovascular disease and breast cancer outweigh the potential benefits of HRT on bone and other tissues. These findings have stimulated the use of alternative therapies for osteoporosis and the search for new and safer pharmacologic agents. MS has two close relatives diagnosed with breast cancer. For this reason, MS should consider an alternative to HRT for treatment of her osteoporosis.

## Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are a group of compounds that bind to the estrogen receptor and have tissue-selective effects on the target organs of estrogen. Depending on the tissue, SERMs are capable of acting as an estrogen agonist or an estrogen antagonist. The mechanisms by which a compound can have both estrogenic and antiestrogenic effects are beginning to be understood. It appears that SERM-ER complexes bind selectively to tissue-specific hormone response elements and/or that SERM-ER complexes recruit tissue-selective transcriptional co-repressors and co-activators (see Chapter 27).

The goal of SERM development is to retain the beneficial effects of estrogen in one or more tissues, while eliminating the undesirable effects of estrogen in other tissues. SERMs are promising agents for the prevention and treatment of postmenopausal osteoporosis. **Raloxifene,** for example, is an estrogen agonist in bone, but an estrogen antagonist in the endometrium and breast (Fig. 29-9). This agent has been approved for the prevention and treatment of osteoporosis. Raloxifene has been demonstrated to increase both vertebral and nonvertebral bone mineral density, and to decrease vertebral fracture risk. Conclusive evidence of its effect on nonvertebral fractures (such as hip fractures) remains to be established. The use of raloxifene is not associated with breast or endometrial cancer. In fact, there is accumulating evidence that raloxifene reduces the risk of breast cancer. Furthermore, raloxifene lowers LDL cholesterol, and may therefore have a role in the prevention of heart disease. However, like estrogen, raloxifene does increase the risk of venous thromboembolism.

*Raloxifene may be the preferred therapy for osteoporosis in women with breast cancer, women with a past history or family history of breast or endometrial cancer,* or women who wish to avoid side effects such as vaginal bleeding and breast tenderness that can be associated with HRT. Because of her family history of breast cancer, MS could especially benefit from raloxifene.

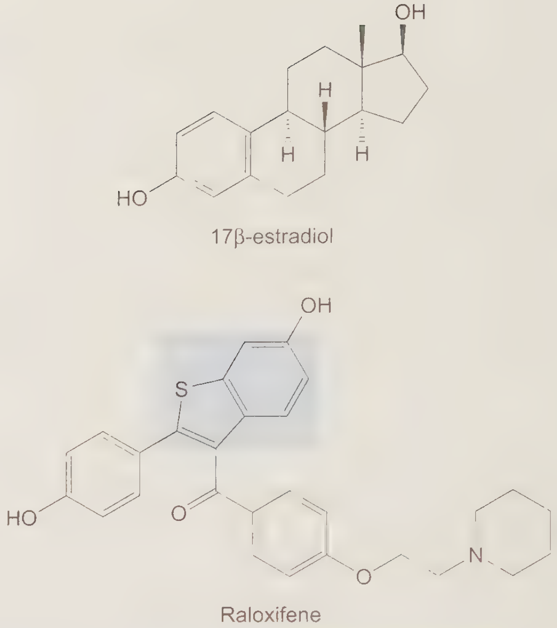
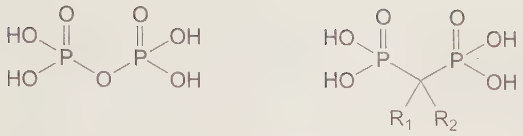


**Figure 29-9. Structures of 17β-Estradiol and Raloxifene.** Although raloxifene is not a steroid molecule, it is conformationally similar to 17β-estradiol. Raloxifene binds to the ligand binding domain on the estrogen receptor, allowing it to act as a partial estrogen agonist in some tissues (bone) and as an estrogen antagonist in other tissues (endometrium and breast). This selective action occurs because the raloxifene–estrogen receptor complex can recruit either transcriptional co-activator or co-repressor factors in a tissue-specific manner (see Chapter 27, Pharmacology of Reproduction, for further details). The benzothiophene nucleus of raloxifene is highlighted in a blue box.

## Bisphosphonates

Bisphosphonates (BPs), currently the most widely used class of antiresorptive drugs, are analogues of pyrophosphate in which the P-O-P bond has been replaced by a nonhydrolyzable P-C-P bond (Fig. 29-10). The three most widely used bisphosphonates—**alendronate, risedronate,** and **pamidronate**—all contain an amine moiety in one of the side chains, which greatly enhances the activity of these so-called amino-bisphosphonates. Because each oxygen in the phosphonate groups can coordinate with a divalent cation like calcium, BPs tend to concentrate in bone, where they are incorporated into the mineralized matrix. BPs remain in the matrix until the bone is subsequently remodeled, at which time the acids secreted by osteoclasts dissolve the mineral matrix and release the BPs.

Some of the released BP molecules are then internalized by the highly phagocytic osteoclasts. Within the osteoclasts, the amino-bisphosphonates inhibit a step in the **mevalonate pathway,** which is involved in protein prenylation. (Prenylation, which includes farnesylation and geranylgeranylation, involves the postranslational addition of lipids to certain proteins, such as GTPases, involved in cellular signaling.) Disruption of the mevalonate pathway leads to the loss of a number of osteoclastic functions, including inhibition of the $H^+$-ATPase, and ultimately leads to osteoclast apoptosis. Because osteoclastic bone resorption increases the local concentration of BP in the vicinity of the osteoclasts, the cellular



Pyrophosphonic acid Bisphosphonic acid

**Figure 29-10. Structures of Pyrophosphate and Bisphosphonate.** Note that the P-O-P structure of pyrophosphate (the protonated form of which is pyrophosphonic acid) is replaced with a P-C-P structure in bisphosphonate (the protonated form of which is bisphosphonic acid). This motif is conserved across all the bisphosphonates. The R-groups differ among the different bisphosphonates; those with an amine moiety have greater potency (not shown).

effects of mevalonate pathway inhibition seem to be limited to osteoclasts. BPs also decrease the solubility of hydroxyapatite, rendering it more resistant to osteoclastic resorption.

The antiresorptive effects of BPs allow these agents to be used in the treatment of osteoporosis, hypercalcemia of malignancy, and Paget's disease. Alendronate and risedronate are the two BPs that are currently approved for the treatment and prevention of osteoporosis. Both oral agents have been shown in clinical trials to significantly increase spine and hip bone mineral density in osteopenic postmenopausal women. In established osteoporosis, both alendronate and risedronate have been shown to decrease the risk of vertebral and nonvertebral fractures. Therefore, oral BPs would be a reasonable therapeutic option for MS. A once-a-week formulation of alendronate improves patient convenience and compliance.

Hypercalcemia of malignancy occurs when certain tumors produce parathyroid hormone-related peptide (PTHrP). This peptide is structurally and functionally related to PTH, and can cause hypercalcemia by the same mechanism as PTH. Intravenous pamidronate and **zolendrolate** have been approved for the treatment of this condition; these agents rapidly inhibit the accelerated bone resorption that results from PTHrP-mediated osteoclast hyperactivity.

BPs have been shown to decrease bone turnover rates in Paget's disease and, thereby, to decrease the progressive deformity, pain, and fractures that are characteristic of this disease.

Because of low oral bioavailability, BPs should be administered upon waking in the morning or on an empty stomach with substantial water. Oral BPs can cause local esophagitis and esophageal erosion; for this reason, patients are advised to remain in the upright position for 30 minutes after taking the medication, and then to have a meal before lying down. The long-term safety of the marked reduction in bone turnover produced by BPs remains to be determined.

## Calcitonin

As discussed above, calcitonin binds to and activates its G protein-coupled receptors on osteoclasts, thereby decreasing the resorptive activity of these cells. Therefore, calcitonin has been used pharmacologically in the treatment of conditions characterized by high osteoclastic activity, such as Paget's disease, osteoporosis, and hypercalcemia. The clinically used preparation of calcitonin is derived from salmon, because salmon calcitonin has a higher affinity for the human calcitonin receptor and a longer half-life than human calcitonin. Because it is a peptide, calcitonin cannot be taken orally, and is instead administered subcutaneously or as a nasal spray. An important drawback to long-term calcitonin administration is the tachyphylaxis that may result from desensitization of the receptor-signaling pathway.

In clinical trials, intranasal calcitonin has been shown to retard vertebral bone loss and to decrease the incidence of vertebral fractures in postmenopausal women. Although the efficacy of calcitonin is lower than that of raloxifene or bisphosphonates, calcitonin can be used as an alternative to these agents in patients who are unable or unwilling to take them. Calcitonin also has weak analgesic properties, possibly because of its effects on endorphin levels. Calcitonin would be a therapeutic option for MS, but its lower efficacy relative to raloxifene would make it a second-line agent.

# BONE ANABOLIC AGENTS

As noted above, antiresorptive agents slow the rate of bone loss but do not build new bone. For patients who have already lost a large amount of bone (BMD more than 2.5 standard deviations below normal) or who have already experienced an osteoporotic fragility fracture, antiresorptive agents are not optimal therapies. This has led to the development of bone anabolic agents, which are drugs that actually increase bone mass, not just prevent its loss. The first bone anabolic agent was fluoride, and the second, more promising, agent is parathyroid hormone.

## Fluoride

Fluoride is known to be a mitogen for osteoblasts. Clinical studies have shown that fluoride, at concentrations higher than those used to fluoridate public water systems, increases trabecular bone mass. Use of fluoride, however, leads to the conversion of hydroxyapatite to fluoroapatite, which is denser but more brittle. Whether fluoride is effective in preventing osteoporosis-related fractures remains uncertain; to date, studies have shown inconsistent results.

## Parathyroid Hormone

As noted above, a persistently elevated plasma concentration of PTH, such as occurs in hyperparathyroidism, leads to increased bone remodeling, with more bone resorbed than formed. As a result, bone can become weak and susceptible to fracture and osteitis fibrosa cystica. In contrast, intermittent stimulation of bone cells by PTH also increases bone remodeling, but with more new bone formed than old bone resorbed. The increase in bone formation occurs because PTH acutely promotes osteoblast development and stimulates osteoblast activity; in comparison, at high or continuous exposure to PTH, the osteoclast-promoting activity of the hormone predominates. Thus, *once-daily subcutaneous administration of PTH favors bone anabolism, while high continuous exposure to PTH favors bone catabolism.*

Native PTH is an 84-amino acid peptide, but N-terminal fragments containing the first 31–34 amino acids of PTH retain all the important functional characteristics of the native protein. Thus, the 1–34 fragment has been shown in clinical trials to act as a powerful anabolic agent that builds new bone. As a peptide, **PTH(1–34)** cannot be administered orally. The currently available formulation is self-injected subcutaneously; whether the peptide could be formulated in an alternative dosage form (e.g., pulmonary) remains to be determined. PTH(1–34) was approved by the FDA in November 2002 for the treatment of both postmenopausal osteoporosis and osteoporosis in men.

## ORAL CALCIUM

Oral calcium has both therapeutic and prophylatic utility. It is administered as a therapy for hypocalcemic states associated with disorders such as vitamin D-dependent rickets and hypoparathyroidism. In severe cases of hypocalcemia, calcium can be administered intravenously. Commonly used intravenous formulations include calcium gluconate and calcium chloride. **Calcium gluconate** is often the preparation of choice, because it produces less venous irritation than the alternatives.

As a preventative measure against osteoporosis or in cases of mild hypocalcemia, calcium is typically administered orally as a calcium salt. Commonly used oral formulations include calcium citrate, calcium carbonate, calcium phosphate, and calcium lactate. Calcium citrate is the most readily absorbed form, but **calcium carbonate** is the most widely used because of its low cost, wide availability (e.g., *Tums*) and antacid properties.

Dietary calcium supplementation has been shown in clinical trials to reduce vertebral bone loss modestly in postmenopausal women, although its effects on fracture prevention are less clear. Because women lose about 15% of skeletal calcium after menopause, prophylactic calcium supplementation in the premenopausal period may help maintain bone density above the critical fracture threshold even in the face of rapid postmenopausal bone loss.

If MS had taken calcium regularly in the premenopausal and perimenopausal periods, it could have helped reduce her risk of a spine fracture. Because she has no history of kidney stones, she should now be counseled to take daily calcium (plus vitamin D) supplementation as part of her therapy for osteoporosis.

## VITAMIN D

Vitamin D preparations include ergocalciferol (also known as vitamin $D_2$), calcifediol (25(OH)D), and calcitriol [1,25 $(OH)_2D_3$]. Synthetic vitamin D analogues include **dihydrotachysterol** (DHT, not to be confused with dihydrotestosterone) and **1α-hydroxycholecalciferol** [$1(OH)D_3$]. DHT is a reduction product of vitamin D; it undergoes 25-hydroxylation in the liver to form 25-hydroxydihydrotachysterol [25(OH) DHT], which is the active form in both bone and intestine. $1(OH)D_3$ is also activated by 25-hydroxylation in the liver. *Both of these synthetic analogues bypass the need for 1-hydroxylation in the kidney and, therefore, are useful in the treatment of bone diseases complicated by renal failure.*

Vitamin D and its analogues are used in the treatment of hypoparathyroidism, rickets, osteomalacia, osteoporosis, and chronic renal failure. When rapidity of action is desired, $1,25(OH)_2D_3$ is preferred because this active form of the vitamin is capable of raising plasma calcium concentrations within 24 to 48 hours. Because vitamin D and its analogues increase both plasma calcium and plasma phosphate, plasma levels of these minerals should be monitored carefully.

In the case of *hypoparathyroidism,* large doses of vitamin D and calcium are used to restore normocalcemia and normophosphatemia. Calcitriol can be administered for more rapid onset of action, but probably does not offer an advantage over vitamin D for long-term treatment.

*For nutritional rickets,* vitamin D is used at low doses as a preventive measure and at higher doses as a treatment. In vitamin D-resistant rickets accompanied by hypophosphatemia, both oral phosphate and high doses of vitamin D are administered; calcitriol has also proved to be useful. For type I vitamin D-dependent rickets, either vitamin D or calcitriol can be used. Type II vitamin D-dependent rickets is refractory to treatment with conventional vitamin D, although high doses of calcitriol have proven to be effective for some patients with this disease.

Conclusive evidence regarding the effectiveness of vitamin D and its analogues as monotherapy in the prevention or treatment of osteoporosis has yet to be obtained. Vitamin D and dietary calcium supplements are used in combination to prevent as well as treat *osteoporosis,* possibly because many elderly individuals have poor calcium intake and are also vitamin D-deficient. The combination has been demonstrated to be modestly efficacious in preventing fracture at vertebral sites, while evidence for the antifracture efficacy at nonvertebral sites is lacking.

In *chronic renal failure,* $1,25(OH)_2D_3$ is used to reverse hypocalcemia and to relieve secondary hyperparathyroidism and osteitis fibrosa cystica. DHT can also be used, although its onset of action is slower. Neither drug corrects the osteomalacia component of chronic renal failure, and patients with hypercalcemia should avoid all forms of vitamin D. 25(OH)D requires several weeks for its actions to become manifested in chronic renal failure, and a high dose of 25(OH)D is required to achieve any clinical effect, possibly because of the reduction in the kidney's ability to convert 25(OH)D to $1,25(OH)_2D$. Vitamin D analogues should not be administered until hyperphosphatemia has been controlled, because the addition of vita- min D analogues can cause increased plasma levels of both calcium and phosphate, leading to metastatic calcification. Control of plasma phosphate levels is typically achieved with dietary phosphate restriction and oral phosphate binders, such as calcium acetate or **aluminum hydroxide.**

## DRUGS AFFECTING PLASMA CALCIUM CONCENTRATIONS

### Hypercalcemia and Its Treatment

Such granulomatous diseases as tuberculosis and sarcoidosis can cause hypercalcemia because of excessive ectopic calcitriol production by mononuclear cells. The resulting increase in calcium absorption by the GI tract can be blunted by the administration of **oral phosphate,** which forms insoluble complexes with dietary calcium and, thereby, decreases calcium absorption. Glucocorticoids (most commonly, prednisone) can be administered as well, to decrease the ectopic production of calcitriol.

The hypercalcemia of malignancy (e.g., breast cancer metastatic to bone) is most commonly treated by one or more of three different approaches: decreasing intestinal calcium absorption, increasing urinary calcium excretion, and inhibiting bone resorption.

For acute hypercalcemia, first-line therapy consists of **saline diuresis.** In this treatment, intravenous saline is administered together with a loop diuretic that increases calcium excretion, such as furosemide. Saline diuresis is very effective in reducing elevated plasma calcium levels rapidly. The saline bolus rehydrates the patient, and ensures adequate renal filtration. Calcium reabsorption in the kidney is in part passive, and is driven by the electrochemical gradient formed by sodium reabsorption. By inhibiting sodium reabsorption, loop diuretics decrease calcium reabsorption and, thus, increase urinary calcium excretion.

Long-term management of hypercalcemia can be achieved using **bisphosphonates** or **calcitonin.** BPs decrease plasma calcium levels by inhibiting osteoclast activity. Several BPs are effective for this purpose, but pamidronate is the first-line therapy. Calcitonin also lowers plasma calcium by its inhibitory effect on osteoclasts. As noted above, the effects of calcitonin are rapid, but limited in duration because of the development of tachyphylaxis within several days.

Severe or symptomatic acute hypercalcemia (plasma calcium >12 mg/dL) is generally treated by a combination of all three of the above approaches: saline diuresis with furosemide, calcitonin, and pamidronate. The first two agents are typically effective within the first 24 hours; whereas, pamidronate is typically effective by the third day.

## Hypocalcemia and Its Treatment

Hypocalcemia can result from a number of underlying pathophysiologies, and treatment is best implemented after the etiology has been identified. For example, hypomagnesemia, which induces target-organ resistance to PTH and decreases PTH synthesis, is a common nutritional cause of hypocalcemia. In this case, treatment with **magnesium sulfate** will ultimately increase plasma calcium. In cases where the plasma magnesium concentration is within normal limits, the treatment of hypocalcemia consists principally of oral calcium and vitamin D (either calcitriol or ergocalciferol).

## *Conclusion and Future Directions*

Bone is composed of organic and inorganic components. The organic component consists of cells (osteoblasts, osteoclasts, and osteocytes) and an organic matrix called osteoid (mainly type I collagen). The inorganic component consists primarily of the calcium phosphate salt hydroxyapatite. The dynamic structure of bone depends on the relative balance between anabolic and catabolic processes, and on the physiologic regulators of calcium and phosphate homeostasis. Three important modulators of bone remodeling and bone mineral homeostasis are parathyroid hormone (PTH), vitamin D, and calcitonin. Through their actions on bone, kidney, and intestine, these three hormones preserve bone mineral homeostasis, sometimes at the expense of bone integrity. Bone disorders can result from abnormal levels of these hormones (e.g., high levels of PTH in hyperparathyroidism, low levels of vitamin D in rickets), increased rates of bone remodeling (e.g., increased bone resorption in osteoporosis, increased formation of disorganized bone in Paget's disease), or failure of organs that are important in maintaining mineral homeostasis (e.g., chronic renal failure). Bone disorders usually lead to structurally weakened bone, because of either: (1) a reduction in bone mass and quality as a result of increased bone resorption; or (2) formation of an architecturally unsound bone. In turn, structural weakening of the bone predisposes to bone fracture or deformity. Bone disorders can be treated by correcting the underlying hormonal or mineral imbalances (e.g., vitamin D, calcium), or by modulating bone remodeling (e.g., SERMs, bisphosphonates). Pharmacologic interventions directed at the physiology of bone remodeling can be divided into two main categories: antiresorptive agents and bone anabolic agents. The majority of drugs currently approved by the FDA for the treatment of osteoporosis are antiresorptive agents. These drugs act by inhibiting osteoclastic bone resorption, thus, slowing down the loss of bone mass. However, these drugs do not stimulate new bone formation and *do not increase true bone mass (matrix plus mineral).* Hence, antiresorptive agents do not represent optimal therapy for individuals who have already sustained significant loss of bone mass. Bone anabolic agents under development, such as the recently FDA-approved once-daily PTH(1–34), act by increasing bone formation and, thus, promise to be the most beneficial agents for patients with very low bone mass.

## *Suggested Readings*

Anonymous. Drugs for the prevention and treatment of postmenopausal osteoporosis. Medical Letter on Drugs and Therapeutics 2000;42:97–100. (*A succinct summary of currently approved drugs*)

Compston J. The role of SERMs in the management of postmenopausal osteoporosis. J Endocrinol Invest 1999;22:646–652. (*Clinical aspects of SERM pharmacology*)

Fiorelli G, Brandi ML. Skeletal effects of estrogens. J Endocrinol Invest 1999;22:589–593. (*Reviews the indices for bone mass and mechanisms of estrogen in controlling bone mineralization*)

Manolagas SC. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000;21:115–137. (*In-depth analysis of the signaling relationships between osteoblasts and osteoclasts*)

Osborne K, Zhao H, Fuqua SAW. 2000. Selective estrogen receptor modulators: Structure, function, and clinical use. J Clin Oncol 2000;18:3172–3186. (*Molecular discussion of SERM action*)

Reid IR. Pharmacotherapy of osteoporosis in postmenopausal women: Focus on safety. Expert Opin Drug Saf 2002;1:93–107. (*Current assessment of therapies for osteoporosis with an emphasis on safety*)

Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science 2000;289:1508–1514. (*Discussion of current and future pharmacologic targets*)

Rubin MR, Bilezikian JP. The anabolic effects of parathyroid hormone therapy. Clin Geriatr Med 2003;19:415–432. (*Current understanding of anabolic actions of PTH*)

Slatopolsky E, Brown A, Dusso A. Pathogenesis of secondary hyperparathyroidism. Kidney Int 1999;5614–19. (*Detailed discussion of chronic renal failure pathophysiology*)

**Drug Summary Table** **Pharmacology of Bone Mineral Homeostasis**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **HORMONE REPLACEMENT THERAPY**<br>*Mechanism—↑ osteoclast apoptosis, ↑ osteoblast lifespan* | | | | |
| **Estrogen + progestin** | Osteoporosis | ↑ Incidence breast cancer<br>↑ Cardiovascular risk<br>Breast tenderness<br>Uterine bleeding<br>Venous thromboembolism | Pregnancy<br>History of thromboembolic disease<br>History or family history of breast cancer | See Chapter 27 for details on estrogens and progestins<br>Women's Health Initiative study concludes risks exceed benefits |
| **SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMs)**<br>*Mechanism—Estrogen receptor partial agonist/antagonist* | | | | |
| **Raloxifene** | Osteoporosis prevention and treatment | Hot flashes<br>Venous thromboembolism | Pregnancy | Estrogen agonist in bone, antagonist in breast and endometrium<br>See Chapter 27 for other SERMs |
| **BISPHOSPHONATES**<br>*Mechanism—Inhibit mevalonate pathway → osteoclast apoptosis, decreased solubility of hydroxyapatite* | | | | |
| **Alendronate**<br>**Risedronate**<br>**Pamidronate**<br>**Zolendronate** | Osteoporosis prevention and treatment<br>Paget's disease<br>Hypercalcemia of malignancy | Esophageal pain and erosion<br>Acid reflux | Delayed esophageal emptying<br>Must remain upright for 30 minutes after oral administration | Long-term effects of profound inhibition of bone turnover unknown<br>Zolendronate only administered IV |
| **CALCITONIN**<br>*Mechanism—↓ osteoclast resorptive activity* | | | | |
| **Salmon calcitonin** | Osteoporosis<br>Hypercalcemia<br>Paget's disease | Facial flushing<br>Nausea<br>Diarrhea, anorexia | Plicamycin may enhance hypocalcemic effect | Salmon calcitonin has greatest efficacy<br>Nasal spray used most widely<br>Weak analgesic activity |

| | | | | |
|---|---|---|---|---|
| **BONE ANABOLIC AGENTS**<br>*Mechanism—↑ osteoblast differentiation and activity* | | | | |
| **Once-daily PTH** | Osteoporosis | Osteosarcoma in long-term rat studies | Paget's disease | Marked increase in bone formation and decrease in vertebral and nonvertebral fractures |
| **Fluoride** | Investigational | Undetermined | Brittle bone | |
| **CALCIUM**<br>*Mechanism—Essential for bone mineralization* | | | | |
| **Calcium gluconate**<br>**Calcium carbonate** | Vitamin D-dependent rickets<br>Hypoparathyroidism<br>Hypocalcemia<br>Osteoporosis | Headache<br>GI disturbance<br>Renal calculi | Renal impairment or calculi<br>Hypercalcemia<br>Hypophosphatemia | Calcium gluconate is IV<br>Calcium carbonate is oral<br>Often used as supplement with vitamin D |
| **VITAMIN D PREPARATIONS**<br>*Mechanism—↑ intestinal calcium absorption* | | | | |
| **Ergocalciferol**<br>**Calcifediol**<br>**Calcitriol**<br>**Dihydrotachysterol**<br>**1α-hydroxycholecalciferol** | Hypoparathyroidism<br>Rickets<br>Osteomalacia<br>Osteoporosis | Headache<br>GI disturbance<br>Renal impairment | Hypercalcemia<br>Renal calculi<br>Hypophosphatemia<br>Hyperparathyroidism | Calcitriol, dihydrotachysterol, and 1α-hydroxycholecalciferol all bypass need for renal hydroxylation |

# V

# Principles of Chemotherapy

# 30

# Principles of Antimicrobial and Antineoplastic Pharmacology

Heidi Harbison, Donald M. Coen, and David E. Golan

**Introduction**
**Mechanisms of Selective Targeting**
- Unique Drug Targets
- Selective Inhibition of Similar Targets
- Common Targets

**Pathogens, Cancer Cell Biology, and Drug Classes**
- Bacteria
- Viruses
- Fungi and Parasites
- Cancer Cells
  - *Carcinogenesis and Cell Proliferation*
  - *Chemotherapy*
  - *Log Cell Kill Model*

**Mechanisms of Drug Resistance**
- Genetic Causes of Drug Resistance
  - *Reduced Intracellular Drug Concentration*
  - *Altered Target*
  - *Insensitivity to Apoptosis*
- Nongenetic Causes of Treatment Failure

**Methods of Treatment**
- Combination Chemotherapy
- Prophylactic Chemotherapeutics

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Although infectious diseases and cancers have different underlying etiologies, from a pharmacologic perspective, the broad principles of treatment are similar. Both sets of diseases are among the most deadly afflictions plaguing human societies. The World Health Organization has estimated that, in the year 2000, infectious diseases caused almost 10 million of the 55 million total deaths worldwide, and malignant neoplasms were responsible for 7 million deaths. Among infectious diseases, the most common causes of mortality worldwide include pneumonia (3.5 million), HIV/AIDS (2.6 million), diarrheal diseases (2.2 million), and tuberculosis (1.6 million). In the developed world, although infectious disease mortality is increasing at a rate of 4.8% per year, cancer (along with heart disease and stroke) is a more significant cause of death. The most deadly cancers in the United States presently include lung cancer (155,000 estimated deaths in 2002), colon cancer (48,000), breast cancer (40,000), prostate cancer (30,000), and pancreas cancer (30,000). Both infectious and neoplastic patterns of disease will likely change, however, as increasingly effective treatments are developed and distributed. The common thread in these pharmacologic strategies is the *targeting of selective differences between the microbe or cancer cell and the normal host cell.* Because both microbes and cancer cells can evolve resistance to drug therapies, the development of new treatments is also a continually evolving process.

Although antimicrobial and antineoplastic drugs are the focus of this chapter, there are many other important and effective strategies to combat microbes and cancer. These strategies include public health measures, vaccinations, and screening procedures. Most public health and vaccination programs aim to prevent infections rather than to treat existing infections. Smallpox, for example, was eradicated worldwide in 1977 through aggressive vaccination programs, although concerns have been raised about the potential use of this virus as a bioterror agent. Similar campaigns to eradicate polio are ongoing. Cancer screening, through regular mammograms, colonoscopy, and other tests, is widely used to detect cancer in its early and more treatable stages. Early detection through the widespread use of Papanicolaou cytologic tests (Pap smears) has caused the mortality of cervical cancer to decrease by two-thirds in the United States; cervical cancer has moved from the primary to the eighth leading cause of cancer deaths in women. Effective strategies against disease, including drug therapy, also depend on socioeconomic factors. In affluent countries, the widespread use of antimicrobial drugs and improvements in sanitation and nutrition have markedly reduced mortality from infectious diseases. This progress has not been realized in the developing world, however, where otherwise treatable infectious diseases such as pneumonia, diarrheal disease, tuberculosis, and malaria remain the predominant causes of mortality.

Despite the importance of public health measures, vaccinations, and screening procedures, drug therapy remains a major tool in the treatment of microbial disease and cancer. The inevitable development of resistance to pharmacologic interventions suggests that an understanding of the general principles and mechanisms of antimicrobial and antineoplastic pharmacology is needed in order to prescribe existing drugs effectively and to continue to discover new drugs.

## MECHANISMS OF SELECTIVE TARGETING

The goal of antimicrobial and antineoplastic drug therapy is **selective toxicity;** i.e., inhibiting pathways or targets that are critical for pathogen or cancer cell survival and replication at concentrations of drug lower than those required to affect host pathways. Selectivity can be realized by: (1) attacking targets unique to the pathogen or cancer cell that are not present in the host; (2) attacking targets in the pathogen or cancer cell that are similar but not identical to those in the host; and (3) attacking targets in the pathogen or cancer cell that are shared by the host, but that vary in importance between pathogen and host and thus, impart selectivity (Table 30-1). These selectively targeted differences can be as great as proteins that are unique to the pathogen, or as slight as the differences in cell cycling and growth rates between some cancer cells and normal cells. In principle, drugs exhibit the least toxicity to the host when they target unique differences and the most toxicity when they target common pathways. For this reason, many antineoplastic drugs are more toxic to the host than are many antimicrobial drugs.

The ratio of the toxic dose to the therapeutic dose of a drug is called the **therapeutic index** or therapeutic window (see Chapter 2, Pharmacodynamics). The therapeutic index is, therefore, an indication of how selective the drug is in producing the desired effects. A highly selective drug, such as penicillin, can often be prescribed safely because of the large difference between its therapeutic and toxic concentrations. The margin of safety in less selective drugs, such as the anticancer drug methotrexate, is much lower because of its low therapeutic index. As more is learned about the biology of pathogens and cancer cells, drugs can be designed against more selective targets. For example, imatinib mesylate is a highly specific anticancer agent that targets the product of a novel gene rearrangement present in chronic myelogenous leukemia cells but not in normal cells (see Chapter 1, Drug–Receptor Interactions). It is important to recognize, however, that many known potential targets remain unexploited because of the toxic side effects, unfavorable pharmacokinetic properties, or prohibitive cost associated with experimental drugs that have been developed to date against these targets.

**TABLE 30-1** Mechanisms of Selective Targeting by Chemotherapeutic Agents

| TYPE OF TARGETING | MECHANISM | EXAMPLE |
|---|---|---|
| Unique | Drug targets genetic or biochemical pathway that is unique to pathogen | Bacterial cell wall synthesis inhibitor |
| Selective | Drug targets protein isoform that is unique to pathogen | DHFR inhibitor |
| Common | Drug targets metabolic requirement that is specific to pathogen | 5-Fluorouracil |

## UNIQUE DRUG TARGETS

Unique drug targets include metabolic pathways, enzymes, and mutated genes and gene products that are present in the pathogen or cancer cell but lacking in the host. One common target for antibacterial drugs is the bacterial peptidoglycan cell wall (see Chapter 35, Pharmacology of the Bacterial Cell Wall). This structure is both biochemically unique and essential for the survival of growing bacteria. Penicillin and other beta-lactam antibiotics inhibit the transpeptidase enzymes that catalyze the final crosslinking step in peptidoglycan synthesis. Without peptidoglycans, bacterial cell wall synthesis is compromised, and cell lysis ensues. Because of their unique specificity for bacterial transpeptidase proteins, the penicillins have minimal host toxicity—in fact, allergic hypersensitivity is the major adverse reaction.

Fungi lack a cell wall and are enveloped only by a lipid bilayer similar to that of human cells, making selective targeting more challenging. Ergosterol, a sterol moiety present in the fungal but not the host membrane, represents one of the few unique targets for antifungal drugs (see Chapter 36, Pharmacology of Fungal Infections). There are currently two classes of drugs that target ergosterol: one class (azoles) blocks ergosterol biosynthesis in fungal cells, and the other class (polyenes) chelates ergosterol in fungal membranes. Both types of drugs alter membrane permeability and cause fungal cell death. Some of these drugs can affect cholesterol metabolism in human cells as well as ergosterol metabolism in fungal cells; these drugs have a low therapeutic index, and their use is associated with significant adverse effects. For example, amphotericin, a polyene used to treat systemic fungal infections, commonly causes fever, rigors, and renal toxicity. Thus, even with a unique target, selectivity can pose a significant challenge.

## SELECTIVE INHIBITION OF SIMILAR TARGETS

Many organisms have metabolic pathways similar to those of humans but, because of evolutionary divergence, possess unique enzyme or receptor isoforms. Drugs can have quantitatively different binding specificities based on these biochemical differences. Because these targets are different but not unique, the resulting therapeutic windows are usually smaller than with unique targets. Examples of this strategy include inhibitors of the enzyme dihydrofolate reductase (DHFR) and inhibitors of bacterial protein synthesis. DHFR is a crucial enzyme in the synthesis of the purine and pyrimidine building blocks of DNA in many organisms (see Chapter 31, Pharmacology of Nucleotide Synthesis). Humans, bacteria, and protozoa all utilize DHFR in DNA synthesis, but the DHFR isoforms are genetically and structurally distinct and can, thus, be targeted by different drugs. The anticancer drug methotrexate powerfully inhibits the DHFR isoforms in human as well as bacterial and protozoal cells, and this drug's poor selectivity produces high toxicity in host cells. The basis for the selectivity of methotrexate in cancer treatment is not a difference in the isoform of the enzyme between cancer cells and normal cells, but rather, the ability of the drug to induce apoptosis in cancer cells but not in most normal cells. In contrast, trimethoprim selectively inhibits the bacterial DHFR and pyrimethamine selectively inhibits the malarial DHFR. Thus, although all these DHFR isoforms bind the same substrate and catalyze the same reaction, biochemical differences in DHFR structure can be exploited for selective inhibition. Determining the amino acid sequence and three-dimensional structure of DHFR isoforms from different species may provide a molecular basis for the rational design of more potent and selective inhibitors in the future.

Bacterial protein synthesis, as with protein synthesis in humans, is a multistep process that involves binding of mRNA to the ribosome, decoding of mRNA, synthesis of peptide bonds, translocation of the polypeptide chain, and release of the polypeptide from the ribosome. The bacterial protein synthesis machinery differs from the human machinery in that different-sized ribosomes and different ribosomal RNAs and proteins are used. Several drug classes, including the macrolides and aminoglycosides, inhibit bacterial protein synthesis (see Chapter 33, Pharmacology of Transcription and Translation). Macrolide antibiotics such as erythromycin bind to the 50S bacterial ribosomal subunit and block the peptide translocation step, preventing emergence of the protein from the ribosome. Aminoglycoside antibiotics such as streptomycin and gentamicin bind to the 30S bacterial ribosomal subunit and disrupt the decoding of mRNA.

The bacterial protein synthesis inhibitors include a wide variety of individual drugs with diverse mechanisms, and the selectivity and dose-limiting toxicities of these drugs are often class- and/or drug-specific. For example, the macrolides rarely cause serious untoward side effects, whereas some of the aminoglycosides have dose-limiting ototoxicity and nephrotoxicity. Some toxic side effects seem to result from drug binding to human mitochondrial ribosomes in addition to bacterial ribosomes. Thus, selective inhibition of similar targets, as exemplified by DHFR inhibitors and protein synthesis inhibitors, can result in effects characterized by therapeutic indices that range from low to high, depending on the individual drug or drug class under consideration.

## COMMON TARGETS

When the host and pathogen or cancer share common biochemical and physiologic pathways, a basis for selectivity may be found if the pathogen or cancer requires a metabolic activity or is affected by its inhibition to a greater degree than the host. These relatively minor differences are often exploited in cancer pharmacology, explaining the narrow therapeutic indices of these drugs. Tumor cells arise from normal cells that have been transformed by genetic mutations into cells with dysregulated growth. These cells utilize the same machinery for growth and replication as do normal cells. Selective inhibition of cancer cell growth is, therefore, a major challenge. Recent discoveries in cancer biology have identified several mutated or overexpressed proteins, inhibitors of which have entered clinical use. The basis for the selectivity of most currently used antineoplastic drugs arises not from biochemical differences, however, but from variations in cancer cell growth behavior and from increased susceptibility of cancer cells to induction of apoptosis. Cancer, as a disease of persistent proliferation, requires continued cell division. Therefore, drugs targeting processes involved in DNA synthesis, mitosis, and cell cycle progression may kill rapidly cycling cancer cells preferentially over their normal relatives. (An important correlate to this statement is that many chemotherapeutic strategies are more successful against rapidly growing than slowly growing cancers.) Antimetabolites such as 5-fluorouracil (5-FU) inhibit DNA synthesis in dividing cells (see Chapter 31). 5-FU inhibits thymidylate synthase, the enzyme responsible for converting dUMP to dTMP, a pyrimidine building block of DNA. As a pyrimidine analogue, 5-FU also incorporates into and interrupts the synthesis of growing RNA and DNA strands. By causing DNA damage, 5-FU induces the cell to activate its apoptotic pathway, resulting in programmed cell death. 5-FU is toxic to all human cells undergoing DNA synthesis, and thus is selectively toxic both for rapidly cycling tumor cells (therapeutic effect) and for such high-turnover host tissues as the bone marrow and GI mucosa (toxic effect).

These examples illustrate the importance of studying the cell biology, molecular biology, and biochemistry of microbes and cancer cells to identify specific targets for selective inhibition. Clinically, an awareness of drug mechanisms and the basis of drug selectivity can help to explain the narrow or broad therapeutic windows that have an impact on drug dosing and treatment strategies. Understanding the selectivity of drugs for their targets is also important in combating drug resistance. Thus, the fundamental pharmacologic principles of drug–receptor interactions, therapeutic and toxic effects, and drug resistance form the basis for selective targeting in antimicrobial and antineoplastic drug therapy.

# PATHOGENS, CANCER CELL BIOLOGY, AND DRUG CLASSES

Pharmacologic interventions target specific differences between the host and the microbial pathogen or transformed cancer cell. This section examines some of the unique characteristics evolution has bestowed on organisms and the major drug classes that target these molecular differences among host cells, pathogens, and cancer cells.

## BACTERIA

Bacteria are prokaryotic organisms that often contain unique targets for pharmacologic intervention. Many of these drug targets are discussed above, and are illustrated in Figure 30-1. Currently available drugs act to interrupt bacterial DNA replication and repair (Chapters 31 and 32), gene expression (Chapter 33), and cell wall synthesis (Chapter 35).

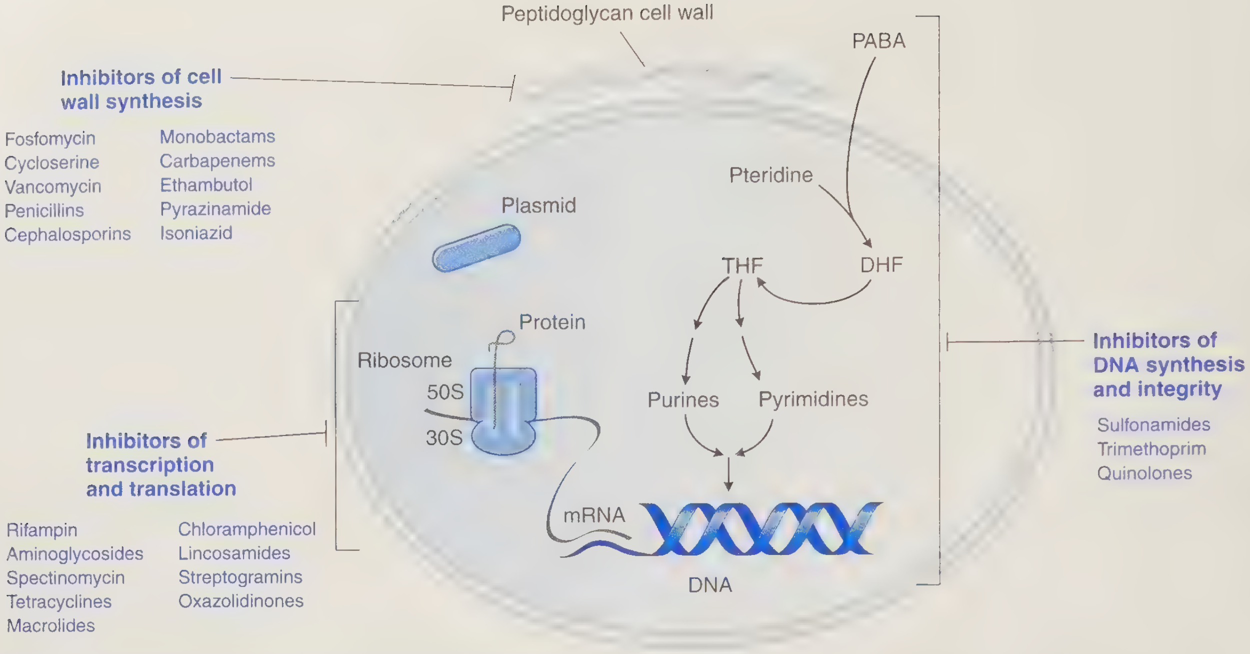

Peptidoglycan cell wall
Inhibitors of cell wall synthesis
Fosfomycin
Cycloserine
Vancomycin
Penicillins
Cephalosporins
Monobactams
Carbapenems
Ethambutol
Pyrazinamide
Isoniazid
PABA
Pteridine
Plasmid
THF
DHF
Protein
Ribosome
50S
30S
Purines
Pyrimidines
Inhibitors of DNA synthesis and integrity
Sulfonamides
Trimethoprim
Quinolones
Inhibitors of transcription and translation
Rifampin
Aminoglycosides
Spectinomycin
Tetracyclines
Macrolides
Chloramphenicol
Lincosamides
Streptogramins
Oxazolidinones
mRNA
DNA

**Figure 30-1. Sites of Action of Antibacterial Drug Classes.** Antibacterial drug classes can be divided into three general groups. Drugs in one group inhibit specific enzymes involved in DNA synthesis and integrity: sulfonamides and trimethoprim inhibit the formation or use of folate compounds that are necessary for nucleotide synthesis; quinolones inhibit bacterial type II topoisomerases. Drugs targeting transcription and translation inhibit bacterial processes that mediate RNA and protein synthesis: rifampin inhibits bacterial DNA-dependent RNA polymerase; aminoglycosides, spectinomycin, and tetracyclines inhibit the bacterial 30S ribosomal subunit; macrolides, chloramphenicol, lincosamides, streptogramins, and oxazolidinones inhibit the bacterial 50S ribosomal subunit. A third group of drugs inhibits specific steps in bacterial cell wall synthesis: fosfomycin and cycloserine inhibit early steps in peptidoglycan monomer synthesis; vancomycin binds to peptidoglycan intermediates, inhibiting their polymerization; penicillins, cephalosporins, monobactams, and carbapenems inhibit peptidoglycan crosslinking; ethambutol, pyrazinamide, and isoniazid inhibit processes necessary for synthesis of the cell wall and outer membrane of *Mycobacterium tuberculosis.* There are several clinically useful antibacterial drugs that do not fit into one of these three groups; one recent example is daptomycin. The development of resistance is a problem for all antibacterial agents. Many bacteria carry plasmids (small, circular segments of DNA) with genes that confer resistance to an antibacterial agent or class of agents. PABA, para-aminobenzoic acid; DHF, dihydrofolate; THF, tetrahydrofolate.

Depending on the role of the drug target in bacterial physiology, antibacterial drugs can produce bacteriostatic or bactericidal effects. Drugs that inhibit the growth of the pathogen without causing cell death are called **bacteriostatic.** These drugs target metabolic pathways that are necessary for bacterial growth but not for bacterial survival. Most protein synthesis inhibitors (aminoglycosides are one exception) have a bacteriostatic effect. The clinical effectiveness of these drugs relies on an intact host immune system to clear the infection. In contrast, **bactericidal** drugs kill bacteria. For example, cell wall synthesis inhibitors (e.g., penicillins and cephalosporins) cause bacterial lysis when the bacteria grow in or are exposed to hypertonic or hypotonic environments. Thus, bacterial infections in immunocompetent hosts can often be treated with bacteriostatic drugs, whereas the treatment of bacterial infections in immunocompromised hosts often requires bactericidal drugs.

Bacteriostatic and bactericidal effects are also important to consider when antibiotics are used clinically in combination. The *combination of a bacteriostatic drug with a bactericidal drug can result in antagonistic effects.* For example, the bacteriostatic drug tetracycline inhibits protein synthesis and, therefore, retards cell growth and division. The action of this drug antagonizes the effects of a cell wall synthesis inhibitor, such as penicillin, which requires bacterial growth to be effective. In contrast, the combination of two bactericidal drugs can be synergistic; that is, the effect of the combination is greater than the sum of the effects of each drug alone (at the same doses of the two drugs). For example, a penicillin-aminoglycoside combination can have a synergistic effect, because inhibition of bacterial cell wall synthesis by the penicillin allows increased entry of the aminoglycoside.

## VIRUSES

Viruses are noncellular organisms that typically consist of a nucleic acid core of RNA or DNA enclosed in a proteinaceous capsid. Some viruses also posses a host cell-derived lipid envelope containing viral proteins. Viruses lack the capability to synthesize proteins themselves, relying instead on the host cell machinery. Because viral replication is dependent on the normal synthetic processes of the host cell, there are fewer antiviral drug classes than antibacterial drug classes, and antiviral drugs are generally more toxic to the host than are antibacterial drugs. Most viruses also encode unique proteins not normally produced by human cells, however. Many of these proteins are involved in the viral life cycle, mediating virus attachment and entry into the host cell, viral capsid uncoating, viral genome replication, viral particle assembly and maturation, and release of viral progeny from the host cell. These virus-specific processes are often targeted by antiviral drugs. A schematic diagram of the general viral life cycle is presented to illustrate the stages of viral replication that can be targeted by antiviral drugs (Fig. 30-2). Because these targets are present only during active viral replication, viruses that exhibit latency are not well controlled by antiviral drugs.

One unique viral protein is the HIV protease. This enzyme cleaves precursor proteins to generate the structural proteins and enzymes necessary for virus maturation. Without HIV protease, only immature and noninfective virions (individual virus particles) are produced. HIV protease inhibitors structurally mimic natural substrates of the protease, but contain a noncleavable bond. These drugs are competitive inhibitors at the active site of the enzyme (see Chapter 34, Pharmacology of Viral Replication). In combination with other classes of anti-HIV drugs, protease inhibitors have revolutionized the treatment of patients with HIV/AIDS.

Several classes of drugs target proteins that are unique to the influenza virus. Zanamivir and oseltamivir target a viral neuraminidase that is vital for virion release from host cells. Amantadine and rimantadine act on the influenza virus membrane protein M2 (a proton channel) to inhibit viral uncoating. Although these anti-influenza drugs are highly effective inhibitors of the viral neuraminidase and proton channel, respectively, they have not revolutionized influenza therapy to the extent that anti-HIV drugs have for HIV. Because most flu infections are identified clinically when the immune system has already begun to eradicate the virus, these drugs have only a limited effect on flu symptoms. This example illustrates the point that even selective inhibitors with high therapeutic indices do not necessarily become highly effective drugs in the clinic.

Currently, the most important antiviral drugs are the polymerase inhibitors. Most viruses use a viral polymerase, either an RNA or DNA polymerase, to replicate their genetic material. Polymerase inhibitors are especially effective against human herpesviruses, HIV, and hepatitis B virus. Two types

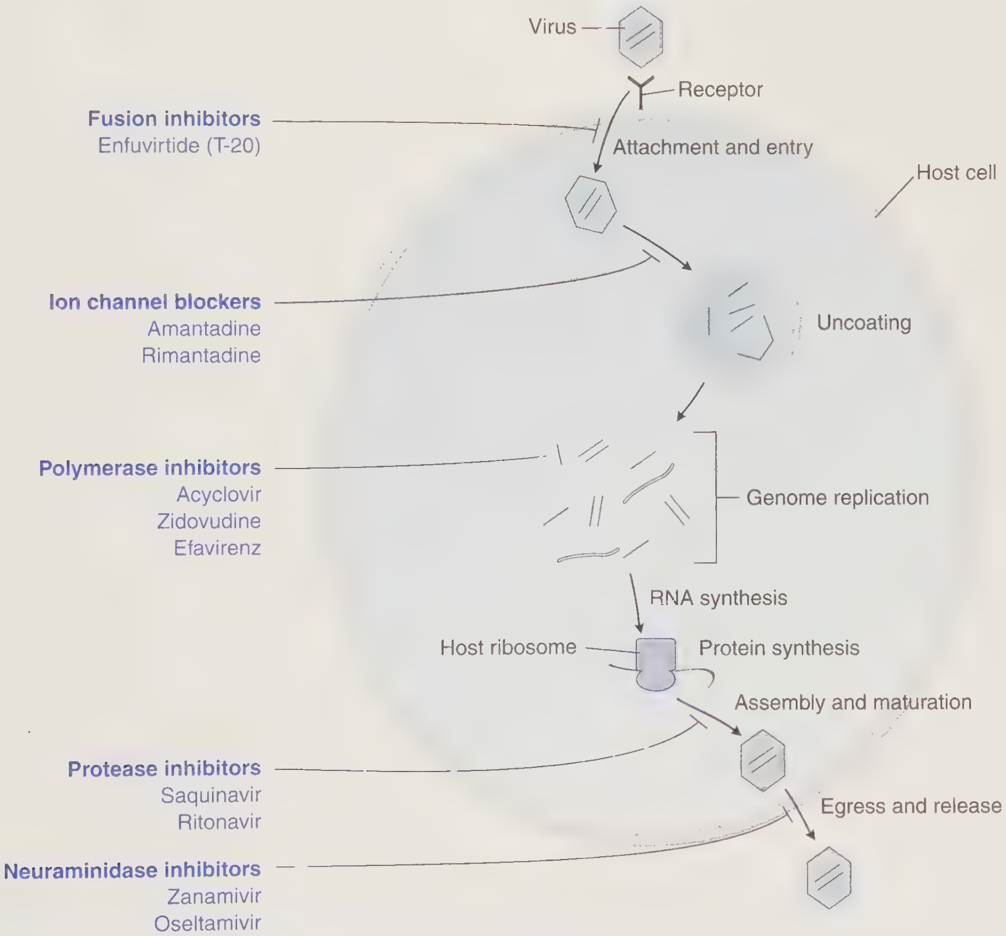

Virus
Receptor
Fusion inhibitors
Enfuvirtide (T-20)
Attachment and entry
Host cell
Ion channel blockers
Amantadine
Rimantadine
Uncoating
Polymerase inhibitors
Acyclovir
Zidovudine
Efavirenz
Genome replication
RNA synthesis
Host ribosome
Protein synthesis
Assembly and maturation
Protease inhibitors
Saquinavir
Ritonavir
Egress and release
Neuraminidase inhibitors
Zanamivir
Oseltamivir

**Figure 30-2. Stages of the Viral Life Cycle Targeted by Antiviral Drug Classes.** The viral life cycle begins with attachment of the virus to a host cell receptor and entry of the virus into the cell. The virus then uncoats, sometimes in an endosomal compartment. The uncoated viral nucleic acid undergoes genome replication; viral genes are transcribed (RNA synthesis); and virally coded RNA is translated into proteins on host cell ribosomes. The replicated viral genome and viral proteins are assembled into a virion (viral particle), which is then released from the host cell. The process of virion assembly and/or release is accompanied by maturation of the virus into an infective agent that is able to repeat this life cycle with a new host cell. The recently approved anti-HIV drug enfuvirtide (T-20) blocks the entry of HIV into host cells. The ion channel blockers amantadine and rimantadine inhibit influenza virus uncoating. Polymerase inhibitors are a large class of antiviral agents that include acyclovir, zidovudine, and efavirenz; these drugs inhibit viral genome replication by interfering with viral DNA polymerase (acyclovir) and reverse transcriptase (zidovudine and efavirenz). Protease inhibitors, such as the anti-HIV drugs saquinavir and ritonavir, inhibit viral maturation. Neuraminidase inhibitors block the release of influenza virus particles from the host cell.

of polymerase inhibitors are the nucleoside analogues and the nonnucleoside reverse transcriptase (NNRT) inhibitors. Nucleoside analogues (such as zidovudine [AZT] and acyclovir) are phosphorylated and, thereby, activated by viral or cellular kinases (phosphorylating enzymes), at which point, they competitively inhibit the viral polymerase and, in some cases, are incorporated into the growing DNA chain. Selectivity is dependent on the relative affinities of the nucleoside analogue for the viral and cellular kinases and polymerases. Nonnucleoside reverse transcriptase inhibitors (such as efavirenz) inhibit viral reverse transcriptase, preventing DNA replication. Chapter 34 provides a detailed discussion of the pharmacology of antiviral drugs. Mutations in viral polymerase genes are a major mechanism for drug resistance.

## FUNGI AND PARASITES

Eukaryotes, which include fungi (yeasts and molds) and parasites (protozoa and helminths) as well as all multicellular organisms, are more complex then prokaryotes. These organisms contain a nucleus and membrane-bound organelles, as well as a cell membrane. Eukaryotic cells reproduce by mitotic division rather than binary fission. Because of the similarities among human, fungal, and parasitic cells, infections caused by fungi and parasites can be more difficult to target than bacterial infections. However, the burden of disease from these organisms is vast. Parasitic infections caused by protozoa and helminths (worms) affect over 3 billion people worldwide, especially in less-developed countries where morbidity and mortality can be devastating. In both developed and less-developed parts of the world, increasing numbers of patients are immunocompromised from AIDS, cancer chemotherapy, organ transplants, and old age. Such patients are especially susceptible to fungal and parasitic infections, which are becoming more prominent and will require greater attention in the future.

The currently available antifungal drugs can be divided into three main classes. As mentioned above, polyenes (amphotericin, nystatin) and azoles (miconazole, fluconazole) selectively target ergosterol in the fungal cell membrane. Pyrimidines such as 5-fluorocytosine inhibit DNA synthesis. Another class of miscellaneous antifungals, mostly acids, are used only topically because of their unacceptable systemic toxicity. As with antibacterials, antifungals can be fungistatic or fungicidal; this distinction is usually determined empirically. For example, the azoles interfere with fungal cytochrome P450-mediated ergosterol metabolism. Many azoles (e.g., itraconazole and fluconazole) are fungistatic. Newer azole agents (voriconazole and ravuconazole) may have fungicidal activity against some fungal species. As compared to fungistatic drugs, fungicidal drugs are more efficacious and faster acting, and allow more favorable dosing regimens. Antifungal drugs are discussed in further detail in Chapter 36.

Parasites exhibit complex and diverse life cycles and metabolic pathways, and the treatment of parasitic infections utilizes a wide array of antiparasitic drugs (see Chapter 37, Pharmacology of Parasitic Infections). Malaria is an example of a complex parasite that, while theoretically susceptible to numerous classes of drugs, is becoming resistant to many currently available therapies. Malaria is transmitted when the female *Anopheles* mosquito deposits *Plasmodia* sporozoites in the human bloodstream. The parasites leave the circulation and develop into tissue schizonts in the liver. The tissue schizonts rupture, releasing merozoites that again enter the circulation to infect red blood cells (erythrocytes). The parasites then mature to trophozoites and finally to mature schizonts. The mature schizonts are released into the bloodstream when the erythrocytes rupture, causing the typical cyclic fever associated with malaria. Antimalarial drugs target different stages of the protozoal life cycle: several classes of drugs can be used, depending on the local pattern of resistance. Aminoquinolines (such as the previous first-line drug, chloroquine) inhibit the polymerization of heme within the erythrocyte; it is thought that nonpolymerized heme is toxic to intraerythrocytic *Plasmodia.* As chloroquine resistance has increased, cinchona alkaloids (quinine and quinidine) and quinoline-methanols (mefloquine) have been used as first-line agents, despite their low therapeutic indices. Dihydrofolate reductase inhibitors, protein synthesis inhibitors, and other classes of drugs are also used in malaria treatment. Malaria is but one example that illustrates the complexities of both the parasitic life cycle and the use of drugs to treat parasitic infections.

## CANCER CELLS

Cancer is a disease of cell proliferation in which normal cells are transformed by genetic mutation into cells with dysregulated growth. Neoplastic cells compete with normal cells for energy and nutrition, resulting in deterioration of normal organ function. Cancers also impinge on vital organs by mass effects. Carcinogenesis, chemotherapy, and the log cell kill model of tumor regression are discussed below to provide an overview of cancer pharmacology. Chapters 31–36 should be read with these principles in mind, and Chapter 38 provides an integrated example of the clinical applications of combination chemotherapy.

### Carcinogenesis and Cell Proliferation

Carcinogenesis occurs in three main steps—transformation, proliferation, and metastasis. **Transformation** denotes a change in phenotype from a cell with normal growth controls to a cell with dysregulated growth. Nonlethal genetic damage (mutations) can be inherited in the germ line, can occur spontaneously, or can be caused by environmental agents such as chemicals, radiation, or viruses. If the DNA damage is not repaired, the mutated genes (e.g., genes involved in growth regulation and DNA repair) can express altered gene products that allow abnormal cell growth and proliferation. Mutations can activate growth-promoting genes, inactivate growth-inhibiting genes, alter apoptosis-regulating genes, confer immortalization, and inhibit DNA repair genes. Expression of altered gene products and/or loss of regulatory proteins can cause genetic instability and dysregulated growth. Most cancers are initially clonal (i.e., genetically identical to a single precursor cell), but evolve to heterogeneity as new mutations increase the genetic variation among daughter cells. When progeny cells with greater survival capacity are selected, increased cell proliferation ensues, and the tumor progresses to greater and greater heterogeneity. Thus, carcinogenesis, the progression from a normal cell to a malignant tumor, is a multistep process that requires an accumulation of multiple genetic alterations. As more is learned about the molecular basis for carcinogenesis, these genetic differences can be targeted for selective drug therapy.

The growth of transformed cells into a tumor requires **proliferation,** or an increase in the number of cells. Dividing human cells progress through a cell cycle (or mitotic cycle) consisting of distinct phases. The two key events in the cell cycle are the synthesis of DNA during S phase and the division of the parent cell into two daughter cells during mitosis or M phase. The phase between cell division and DNA synthesis is called gap 1 (G1), and the phase between DNA synthesis and mitosis is called G2. Proteins called cyclins and cyclin-dependent kinases (CDKs) govern progression through the phases of the cell cycle; mutations in cyclin and/or CDK genes can result in neoplastic transformation.

A proliferating cancer cell has three potential fates: the daughter cell can become quiescent by entering a resting phase called G0; the cell can enter G1 and proliferate; or the cell can die. The ratio of the number of cells that are proliferating to the total number of cells in the tumor is called the **growth fraction.** An average tumor growth fraction is about 20%, because only one in five cells participates in the cell cycle at any given time. Most antineoplastic drugs target dividing cells. Hence, tumor cells in a quiescent (G0) state, such as nutrient-starved cells in the center of a large tumor, are not easily killed by chemotherapy. Small or rapidly growing cancers (i.e., cancers with high growth fractions, such as leukemias) often respond more favorably to chemotherapy than do large bulky tumors. Unfortunately, cells in normal tissues characterized by high growth fractions, such as the bone marrow and GI mucosa, are also killed by antineoplastic drugs, resulting in dose-limiting toxicities.

Tumor cells do not proliferate in isolation. Transformed cancer cells secrete a variety of chemical mediators to induce the creation of a specialized local environment. These chemical mediators include growth factors such as epidermal growth factor (EGF), and inhibitors of growth factor signalling are now in clinical use. Some tumors create a protective fibrous connective tissue stroma. For example, this property makes breast cancer nodules palpable. Most solid tumors also require the induction of blood vessel growth (angiogenesis) to deliver nutrients into the center of the tumor, and angiogenesis inhibitors represent a potentially valuable new class of antineoplastic drugs.

Cancer cells may acquire the capability to invade tissues and **metastasize** throughout the body. To metastasize, tumor cells must acquire mutations that allow invasion into tissues and vessels, seeding of cavities, spread through lymph or blood vessels, and growth in a new environment. Aggressive, rapidly growing primary tumors are generally more likely to metastasize than are more indolent and slowly growing tumors. In the process of gaining mutations, tumor cells can also evolve differential receptor expression patterns and drug sensitivities. Often, although the primary tumor may respond well to chemotherapy, the more highly dedifferentiated metastatic cells respond poorly. Thus, metastatic spread often represents a poor prognostic sign.

## Chemotherapy

By the time a typical solid tumor is clinically evident, it contains at least $10^9$ cells, has progressed to heterogeneity, and has developed surrounding stroma. The tumor may or may not have metastasized from its site of origin ("primary site") to one or more secondary sites. These factors can render the cancer difficult to treat pharmacologically. Currently, most chemotherapeutic agents interfere with cell proliferation and rely on rapid cell cycling and/or promotion of apoptosis for relative selectivity against cancer cells (Fig. 30-3). As noted above, tumors are most sensitive to chemotherapy when they are growing rapidly, primarily because they are progressing through the cell cycle. These *metabolically active cells are thus susceptible to drugs that interfere with cell growth and division* (the **mitotoxicity hypothesis**). Many antineoplastic drugs interfere with the cell cycle at a particular phase; such drugs are called **cell-cycle specific**. Other antineoplastic drugs act independently of the cell cycle and are called **cell-cycle nonspecific** (Fig. 30-4). Inhibitors of DNA synthesis, such as antimetabolites and folate pathway antagonists, are S phase specific. Microtubule poisons, such as taxanes and vinca alkaloids, interfere with spindle formation during M phase. The alkylating agents that damage DNA and other cellular macromolecules act during all phases of the cell cycle. These various classes of drugs can be administered in combination, using cell-cycle specific drugs to target mitotically active cells and cell-cycle nonspecific agents to kill both cycling and noncycling tumor cells (see Chapter 38).

The mitotoxicity hypothesis of anticancer therapy leaves some puzzles unresolved, however. Although cancer chemotherapy is often toxic to the bone marrow, GI mucosa, and hair follicles, these tissues usually recover while (in successful treatment) cancers with similar growth kinetics are eradicated. *It has now been established that almost all chemotherapeutic drugs also cause apoptosis of cancer cells.* DNA damage is normally sensed by molecules, such as p53, that arrest the cell cycle to allow time for the damage to be repaired. If the damage is not repaired, a cascade of biochemical events is triggered, which results in **apoptosis** (programmed cell death). Therefore, a cancer cell that has a defective capability for DNA repair may undergo apoptosis, whereas a normal cell can repair its DNA and recover. Cancers that express wild-type p53, such as most leukemias, lymphomas, and testicular cancers, are often highly responsive to chemotherapy. In contrast, cancers that acquire a mutation in p53, including many pancreatic, lung, and colon cancers, are often minimally responsive or even resistant to DNA-damaging drugs.

## Log Cell Kill Model

The **log cell kill model** is based on experimentally observed rates of tumor growth and tumor regression in response to chemotherapy. Tumor growth is usually exponential, with a doubling time (i.e., time required for the total number of cancer cells to double) that depends on the type of cancer. For example, testicular cancer often has a doubling time of less than 1 month, whereas colon cancer tends to double every 3 months. In solid tumors, cells may grow exponentially until a clinically observable tumor size is achieved. (Typically, a newly detected 1-cm tumor contains about $10^9$ cells.) *The log cell kill model states that cell destruction in cancer chemotherapy is first-order;* that is, that each dose of chemotherapy kills a constant fraction of cells. If the tumor starts with $10^{12}$ cells and 99.99% are killed, then $10^8$ malignant cells will remain. The next dose of chemotherapy will then kill 99.99% of the remaining cells, and so on. Unlike antibacterial drugs, which can be used in a constant high dose until the bacteria are eradicated, antineoplastic drugs must be used intermittently to reduce toxic side effects. Intermittent dosing allows

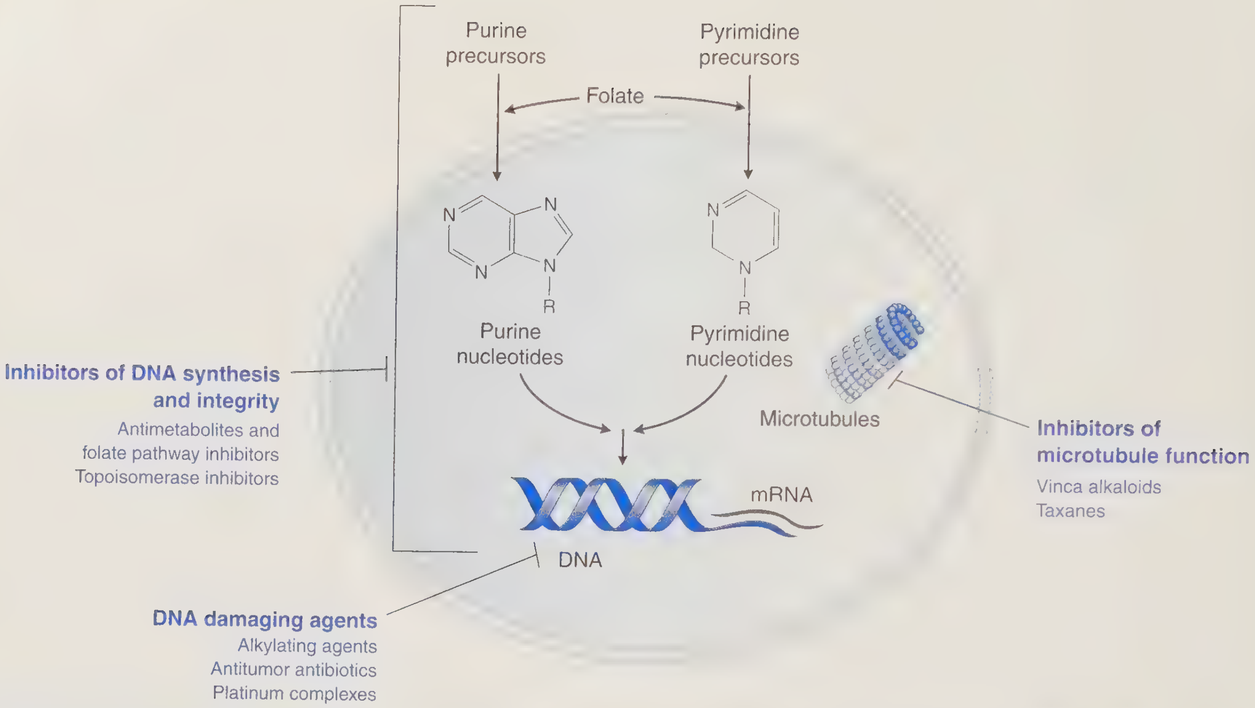


**Figure 30-3. Antineoplastic Drug Classes.** Many cancer cells divide more frequently than normal cells, and cancer cells can often be killed preferentially by targeting three critical processes in cell growth and division. DNA damaging agents alter the structure of DNA and, thereby, promote apoptosis of the cell. These agents include alkylating agents (which covalently couple alkyl groups to nucleophilic sites on DNA), antitumor antibiotics (which cause free radical damage to DNA), and platinum complexes (which crosslink DNA). Inhibitors of DNA synthesis and integrity block intermediate steps in DNA synthesis; these agents include antimetabolites and folate pathway inhibitors (which inhibit purine and pyrimidine metabolism) and topoisomerase inhibitors (which induce damage to DNA during winding and unwinding). Inhibitors of microtubule function interfere with the mitotic spindle that is required for mitosis. This group of drugs includes vinca alkaloids, which inhibit microtubule polymerization, and taxanes, which stabilize polymerized microtubules. Additional classes of antineoplastic agents, such as hormones and monoclonal antibodies, are not shown.

partial recovery of normal cells, but also provides time for cancer cell regrowth and for evolution of drug resistance. As seen in Figure 30-5, intermittent "cycles" of chemotherapy are administered until all the cancer cells are killed or the tumor develops resistance. Drug-resistant cells continue to grow exponentially despite treatment, eventually resulting in death of the host. Improvements in the rates of eradication of malignant cell populations are likely to require either higher doses of chemotherapeutic agents (which are limited by toxicity and resistance) or initiation of therapy at a time when the tumor contains fewer cells (which implies earlier detection). Adjuvant therapies, such as surgery and radiation, are other important modalities used to reduce the number of tumor cells before chemotherapy is initiated. Surgery and radiation may also serve to recruit more tumor cells into the cell cycle and, thus, to increase the susceptibility of these cells to cell-cycle specific agents.

## MECHANISMS OF DRUG RESISTANCE

Having provided a general introduction to the pharmacology of drug targets in bacteria, viruses, fungi, parasites, and cancer, the discussion now turns to the mechanisms of drug resistance, which is a major problem in all of antimicrobial and antineoplastic pharmacology. Although resistance to current drug therapies is emerging relatively rapidly, the rate of introduction of new drugs is relatively slow. Formerly curable diseases such as gonorrhea and typhoid fever are becoming more difficult to treat, and old killers such as tuberculosis and malaria are growing increasingly resistant worldwide. The WHO estimates that 98% of gonorrhea isolates in southeast Asia are multidrug resistant. In the United States, 60% of hospital-acquired (nosocomial) infections are caused by drug-resistant microbes. Tuberculosis, the third leading cause of infectious disease death worldwide, currently has an estimated 2% multidrug resistance (MDR) rate worldwide, although the rate is as high as 15% in some countries such as Estonia. The recent appearance of MDR tuberculosis in the United States is of special concern because of the airborne spread of this organism. Despite these ominous trends, only three new classes of antibiotics—oxazolidinones, streptogramins, and daptomycin—have been developed in the past three decades. The numerous examples of rapidly emerging drug-resistant organisms suggest that this problem must be addressed promptly.

Because pathogens and cancer cells are primed to evolve rapidly in response to adaptive pressure, resistance can eventually appear with the use of any antimicrobial or antineoplastic drug. In a population of microbes or transformed cells, the cells that contain random mutations promoting fitness will survive. Thus, high cell number, rapid growth rate, and high mutation rate all promote the development of a heterogeneous population of cells that can acquire resistance through muta-

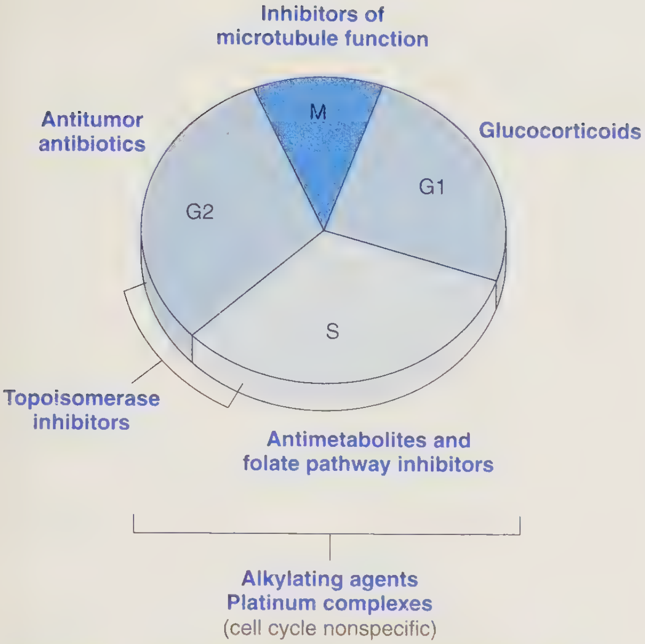


**Figure 30-4. Cell-Cycle Specificity of Antineoplastic Drug Classes.** The cell cycle is divided into four phases. Cell division into two identical daughter cells occurs during mitosis (M phase). Cells then enter the gap 1 (G1) phase, which is characterized by active metabolism in the absence of DNA synthesis. Cells replicate their DNA during the synthesis (S) phase. After completion of S phase, the cell prepares for mitosis during the gap 2 (G2) phase. Antineoplastic drugs exhibit specificity for different phases of the cell cycle depending on their mechanism of action. Inhibitors of microtubule function affect cells in M phase; glucocorticoids inhibit cells in G1; antimetabolites and folate pathway inhibitors inhibit cells in S phase; antitumor antibiotics inhibit cells in G2; topoisomerase inhibitors inhibit cells in S phase and G2. Alkylating agents and platinum complexes affect cell function in all phases, and are, therefore, cell-cycle nonspecific. The differential cell-cycle specificity of the various drug classes allows them to be used in combination to target different populations of cells. For example, cell-cycle specific drugs can be administered to target actively replicating neoplastic cells, while cell-cycle nonspecific agents can be used to target quiescent (nonreplicating) neoplastic cells.

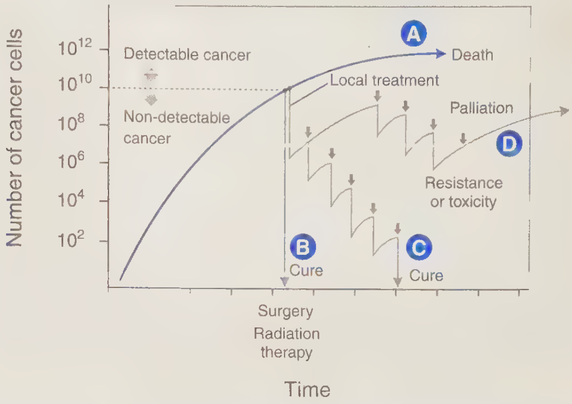


**Figure 30-5. Log Cell Kill Model of Tumor Growth and Regression.** The log cell kill model predicts that the effects of antineoplastic chemotherapy can be modeled as a first-order process. That is, a given dose of drug kills a constant *fraction* of tumor cells, and the number of cells killed depends on the total number of cells remaining. The four curves (A–D) represent four possible outcomes of antineoplastic therapy. Curve A is the growth curve of untreated cancer. The cancer continues to grow over time, eventually resulting in the death of the patient. Curve B represents curative local treatment (surgery and/or radiation therapy) before metastatic spread of the malignancy. Curve C represents local treatment of the primary tumor, followed immediately by systemic chemotherapy administered in cycles (indicated by down arrows) to eradicate the remaining metastatic cancer cells. Note that each cycle of chemotherapy reduces the number of cancer cells by a constant fraction (here, by about two "logs," or by about 99%), and that some cancer growth occurs as the normal tissues are given time to recover between cycles of chemotherapy. Curve D represents local treatment followed by systemic chemotherapy that fails when the tumor becomes resistant to the drugs or when toxic drug effects occur that are intolerable to the patient. Note that approximately $10^{10}$ cancer cells must be present for a tumor to be detectable; for this reason, multiple cycles of chemotherapy are required to eradicate the cancer, even when there is no detectable tumor remaining.

tional escape. Because the use of a drug inherently selects for organisms that can survive in the presence of high concentrations of that drug, resistance is an omnipresent consequence of drug therapy. In many cases, the emergence of drug resistance confounds effective treatment.

## GENETIC CAUSES OF DRUG RESISTANCE

The recent explosion in drug resistance has both genetic and nongenetic causes. Genetic mechanisms of resistance arise from chromosomal mutations and from exchange of genetic material. Chromosomal mutations usually occur in the gene(s) that code(s) for the drug target or in genes that code for drug transport or metabolism systems. These mutations can then be conferred on daughter cells (**vertical transmission**) to create drug-resistant organisms. Alternatively, bacteria can acquire resistance by gaining genetic material from other bacteria (**horizontal transmission**). For example, methicillin-resistant *Staphyloccocus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE) are able to cause two highly feared nosocomial infections, because these bacteria have acquired resistance genes. Bacteria acquire genetic material by three main mechanisms—conjugation, transduction, and transformation. In **conjugation,** chromosomal or plasmid DNA is transferred directly between bacteria. DNA can also be transferred from one cell to another by a bacterial virus, or bacteriophage, in a process called **transduction.** In **transformation,** naked DNA in the environment can be taken up by the bacteria. Drug resistance is most often caused by the transfer of plasmids, which are extrachromosomal strands of DNA that contain drug resistance genes.

Transfer of a DNA plasmid is especially important for drug resistance because this mechanism occurs at high rates both within and between bacterial species, and because multidrug resistance genes can be transferred. Specific mechanisms of resistance for each type of organism are discussed in subsequent chapters. Table 30-2 lists the major mechanisms of genetic drug resistance that can be caused by either chromosomal mutation or genetic exchange.

**TABLE 30-2** Mechanisms of Genetic Drug Resistance

| MECHANISM | EXAMPLE: ANTIMICROBIAL | EXAMPLE: ANTINEOPLASTIC |
|---|---|---|
| **Reduced Intracellular Concentration of Drug** | | |
| Inactivate drug | Inactivation of β-lactam antibiotics by β-lactamase | Inactivation of antimetabolites by deaminase |
| Prevent uptake of drug | Prevention of aminoglycoside entry by altered porins | Decreased methotrexate entry by decreased expression of reduced folate carrier |
| Promote efflux of drug | Efflux of multiple drugs by MDR membrane efflux pump | Efflux of multiple drugs by p170 membrane efflux pump |
| **Altered Drug Target** | Expression of altered peptidoglycan that no longer binds vancomycin | Expression of mutant DHFR that no longer binds methotrexate |
| **Insensitivity to Apoptosis** | N/A | Loss of active p53 |
| **Bypass Metabolic Requirement for Target** | Inhibition of thymidylate synthase bypassed by exogenous thymidine | Loss of estrogen receptor-dependent growth results in tamoxifen resistance |

## Reduced Intracellular Drug Concentration

Drugs must reach their targets in order to be effective. When an insufficient amount of drug reaches the target, the growth of pathogens or tumor cells and the emergence of resistant strains are allowed. Microbes and tumor cells have evolved a number of mechanisms to **inactivate drugs** before the drugs can bind to their targets. Many bacteria are resistant to penicillins and cephalosporins because they produce a hydrolytic enzyme, β-lactamase, that cleaves the β-lactam ring and, thereby, disables the drug's active site. A single β-lactamase enzyme can hydrolyze $10^3$ penicillin molecules per second, significantly reducing the intracellular concentration of active drug. As another example, tumor cells that overexpress a deaminase enzyme can rapidly inactivate purine or pyrimidine analogues (antimetabolites), making these drugs less effective.

Pathogens and cancer cells can also acquire mutations that **prevent uptake of the drug** into the cell or otherwise prevent access of the drug to the target molecule. For example, cancer cells with mutated folate-transport systems become resistant to folate analogues, such as methotrexate, that require active transport into cells in order to inhibit DHFR.

Finally, both bacteria and tumor cells can acquire the ability to cause active **drug efflux** from the cell. Bacteria typically possess membrane pumps to transport lipophilic or amphipathic molecules (such as antibiotics against other bacteria) in and out of the cells. Overproduction of these membrane proteins or their variants can mediate active pumping of a therapeutic antibiotic out of the cell faster than the drug can enter the cell. Despite the achievement of therapeutic blood levels of the antibiotic, this active efflux mechanism can cause intrabacterial drug concentrations to be ineffectively low. Similarly, the emergence of multidrug resistant (MDR) cancers is often associated with tumor cell overexpression of a membrane-bound glycoprotein, the P-glycoprotein (p170 or MDR1), that actively pumps antineoplastic drugs out of the cell. These efflux pumps are especially important because they are capable of pumping out more than one type of drug and, thus, of allowing pathogens or tumors to become resistant to multiple drugs of different classes.

## Altered Target

In addition to chemically destroying or pumping out drugs, cells can also reprogram or camouflage drug targets. Alteration in the target of a drug through mutation of the gene(s) that codes for that target is a common mechanism for development of drug resistance. In vancomycin-resistant enterococcus, the vanHAX genes encode a novel enzymatic pathway that reprograms the surface peptidoglycan so as to terminate in the sequence D-Ala-D-Lactate instead of the normal D-Ala-D-Ala. This substitution does not affect the peptidoglycan crosslinking process in bacterial cell wall synthesis, but it does lower the binding affinity of vancomycin for the dipeptide by 1,000-fold. In cancer cells, both quantitative and qualitative changes in the enzyme targets of antineoplastic drugs—such as DHFR, thymidylate synthase, and topoisomerase—can reduce drug binding (potency) and, thereby, confer drug resistance.

## Insensitivity to Apoptosis

The development of drug resistance in cancer cells occurs through chromosomal mutations that are then passed to daughter cells to create a resistant tumor. Although cytotoxic anticancer drugs act at a variety of molecular targets, most, if not all, ultimately cause cell death by inducing apoptosis. In general, drug-induced **molecular lesions** can lead to cell cycle arrest, activation of repair processes, or apoptosis. Mutations in key proteins associated with the control of apoptosis, such as p53 and Bcl-2, can result in failure to induce the apoptotic response to DNA damage and can, thereby, reduce the sensitivity of tumor cells to anticancer drugs. As noted above, tumors with wild-type p53, such as many leukemias, lymphomas, and testicular cancers, are often highly responsive to chemotherapy. In contrast, many pancreatic, lung, and colon cancers have a high incidence of p53 mutations and are minimally responsive to chemotherapy.

Thus, the genetic causes of drug resistance include both mutations in chromosomal DNA and external acquisition of genetic material. Genetic resistance can be caused by drug inactivation, decreased drug uptake, increased drug efflux, reprogramming of the target structure or pathway, repair of drug-induced lesions, and insensitivity to apoptosis. Resistance is probably the major limiting factor in the effective treatment of both infections and cancer. Drug therapy is a dynamic balance, an "evolutionary arms race," between the design of new drugs and the evolution of changes leading to drug resistance.

## NONGENETIC CAUSES OF TREATMENT FAILURE

The treatment of an infection or cancer can fail for numerous reasons. One of the most important mechanisms of drug resistance is the excessive overprescription of antibiotics that are not indicated for the clinical situation. Overprescription is a problem not only in humans, but also in the treatment and prophylaxis of animal infections. Such widespread use promotes drug resistance, which is then transferred from one bacterium to another by the mechanisms described above. Other mechanisms of resistance involve pharmacologic and anatomical drug barriers, such as the wall of an abscess or the blood–brain barrier. Poor patient compliance can also promote resistance, as can the erratic drug availability found in parts of the developing world (and even in some communities in the developed world). International travel promotes a global disease community, ensuring that the multidrug-resistant tuberculosis found in Russia or Peru will eventually emerge in hospitals in the United States. Finally, demographic shifts and other trends have created large susceptible populations, such as immunocompromised cancer patients, AIDS patients, and the elderly population.

# METHODS OF TREATMENT

## COMBINATION CHEMOTHERAPY

The development of drug resistance depends on such factors as the number of microbial or tumor cells in the pretreatment population, the rate of replication or "generation time" of the pathogenic cell population, and the intrinsic rate of mutation in the population. Compared to treatment with a single agent, treatment with a combination of drugs can significantly decrease the probability that resistance will develop. Combination chemotherapy is now the standard-of-care in HIV therapy and most antineoplastic drug regimens. There are several major reasons to administer multiple drugs simultaneously in a combination chemotherapy regimen; the rationales are discussed in further detail in Chapter 38. First, the use of multiple drugs with different mechanisms of action targets multiple steps in microbial or cancer cell growth, leading to the maximum possible rate of cell killing. Second, the use of combinations of drugs that target different pathways or molecules in the pathogen or cancer cell makes it more difficult for the foreign cell to become resistant to the therapy. Even if the likelihood of development of a resistance mutation to one drug is relatively high, the simultaneous evolution of separate mutations against several different drugs is less likely. Third, the use of lower doses of synergistically acting drugs in the combination can reduce drug-associated toxicity. This is especially important in antimicrobial chemotherapy, where synergistic activity of drug combinations has been clearly demonstrated. Fourth, because many antineoplastic drugs have different dose-limiting toxicities, it is often possible to use each drug to its maximally tolerated dose and, thereby, to achieve increased overall cell killing. Finally, the concept of combination chemotherapy is being redefined as new treatments become available. In the future, immunotherapies, hormone therapies, and biotherapies will become increasingly integrated into combination chemotherapy regimens.

## PROPHYLACTIC CHEMOTHERAPEUTICS

In most instances, antimicrobial and antineoplastic drugs are used to treat overt disease. These classes of drugs can also be used to prevent diseases from occurring (chemoprophylaxis), both before a potential exposure and after a known exposure. The potential benefit of chemoprophylaxis must always be weighed against the risk of creating drug-resistant pathogens or cancer cells and the potential for toxicity attributable to the chemoprophylactic agent. Antimicrobial chemoprophylaxis is frequently used in high-risk patients to prevent infection. Travelers to malaria-infested areas, for example, often take prophylactic antimalarial drugs such as chloroquine or mefloquine. Chemoprophylaxis is also used in some types of surgery to prevent wound infections. Antibiotics are commonly administered prophylactically during surgical procedures that could release bacteria into the wound site, such as colon resection. Antibiotics are also used prophylactically before dental procedures in patients at high risk for endocarditis, because such procedures can produce a transient bacteremia. In certain situations, immunocompromised patients are given antibacterial, antifungal, antiviral, and/or antiparasitic drugs prophylactically to prevent opportunistic infections. For example, acyclovir can protect previously infected immunocompromised patients against disease caused by reactivation of latent herpes simplex virus.

Chemoprophylaxis or pre-emptive therapy can also be used in healthy persons after exposures to certain pathogens. Prophylactic therapy after known or suspected exposure to gonorrhea, syphilis, bacterial meningitis, HIV, and other infections can often prevent disease. The risk of seroconversion after a single needle stick exposure to HIV-infected blood depends on the type of exposure, ranging from 0.1 to 4.5%. Although limited data are available regarding the reduction of risk achievable with prophylaxis, the CDC currently recommends postexposure treatment with zidovudine (AZT) and lamivudine (3TC) for 4 weeks. Zidovudine has also been shown to reduce maternal transmission of HIV, representing chemoprophylaxis for the fetus.

## *Conclusion and Future Directions*

Many of the principles underlying the pharmacologic treatment of microbial diseases and cancer are similar. Pharmacologic treatments of both infection and cancer rely on selective inhibition of the pathogen or cancer cell to prevent its growth or survival, with a minimum of toxic side effects that could interfere with host cell function. Selective inhibition of a unique target, such as the bacterial cell wall, is ideal. Often, less selective therapies, targeting a molecule or pathway that is similar or even identical between the pathogen or cancer cell and the host, must be employed. Even highly selective drugs aimed at an entirely unique target can be rendered ineffective if the microbe or cancer cell mutates to become resistant. Both microbes and cancer cells grow rapidly, with the potential for evolving or acquiring mutations that confer resistance. Physicians attempt to circumvent the development of resistance by initiating treatment early, using maximally tolerated doses of drugs, and administering multiple drugs in combination. Despite these strategies, however, resistance has become a major impediment to successful therapy. As more is learned about the biology of microbes and cancer cells, and more unique targets are discovered, it is hoped that treatments will become more selective, less toxic, and less prone to the development of drug resistance.

## *Suggested Readings*

Cohen ML. Changing patterns of infectious disease. Nature 2000; 406:775–781. (*Documents the rise of drug resistance.*)

Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Disease. 5th ed. Philadelphia: Churchill Livingstone Inc., 2000. (*Authoritative textbook on clinical management of infectious diseases.*)

Morrow CS, Cowan KH. Drug resistance and its clinical circumvention. In: Kufe DW, Pollock RE, Weichselbaum RR, Bast RC Jr, et al. eds. Cancer Medicine. 6th ed. Atlanta: American Cancer Society and BC Decker Inc., 2003. (*In-depth mechanisms of genetic resistance to pharmacologic agents.*)

Walsh CT. Antibiotics: Actions, Origins, Resistance. Washington, DC: ASM Press, 2003. (*Reviews the structural and chemical basis for drug resistance.*)

WHO Statistical information system. www.who.int/whosis/ (*Source of the statistics provided in this chapter.*)

# 31

# Pharmacology of Nucleotide Synthesis

Harris S. Rose and Robert R. Rando

**Introduction**
**Case**
**Biochemistry of Nucleotide Metabolism**
- Nucleotide Synthesis
  - *Purine Ribonucleotide Synthesis*
  - *Pyrimidine Ribonucleotide Synthesis*
  - *Ribonucleotide Reduction and Thymidylate Synthesis*
  - *Folate Metabolism*
- Nucleic Acid Synthesis

**Pharmacologic Classes and Agents**
- Inhibitors of Folate Metabolism
  - *Inhibitors of Dihydropteroate Synthase*
    - *Sulfonamides*
    - *Sulfones*
  - *Inhibitors of Dihydrofolate Reductase*
    - *Methotrexate*
    - *Trimethoprim*
    - *Pyrimethamine*
    - *Synergy of DHFR Inhibitors and Sulfonamides*
- Inhibitors of Thymidylate Synthase
- Inhibitors of Purine Metabolism
- Inhibitors of Ribonucleotide Reductase
- Purine and Pyrimidine Analogues That Are Incorporated into DNA

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Chapter 30, Principles of Antimicrobial and Antineoplastic Pharmacology, introduces the concept of cell growth and division as a chemotherapeutic target. This chapter and the next discuss drugs that inhibit the cellular machinery involved in cell growth and division. This chapter focuses on the **antimetabolites,** which are analogues of endogenous folates, purines, and pyrimidines that act mainly to inhibit the enzymes of nucleotide synthesis. Nucleotides are the molecular building blocks of the nucleic acids DNA and RNA. Without effective nucleotide synthesis, a cell cannot replicate its genetic material; therefore, antimetabolites act specifically in the DNA synthesis (S) phase of the cell cycle. Pharmacologic strategies that interrupt DNA replication and mitosis, as well as drugs that directly damage the structure of DNA, are discussed in the next chapter.

The exceptional therapeutic utility of the antimetabolites is demonstrated by their successful application to nearly all forms of chemotherapy: antibacterial, antifungal, antiparasitic, and antineoplastic. In part, the antimetabolites have broad applicability because their clinical effectiveness is linked to their selectivity. For example, an antimetabolite can be used as an antibiotic, taking advantage of a bacterial requirement for a specific enzyme that is not expressed in human cells; a related drug can be used as a cancer chemotherapeutic agent, because of the elevated requirement of rapidly dividing cancer cells for nucleotide synthesis. Antimetabolites are also used as immunosuppressive agents; this specialized application is discussed separately in Chapter 43, Pharmacology of Immunosuppression.

## Case

The country is Germany, and the year is 1935. Hildegard, daughter of Dr. Gerhard Domagk, is near death with a streptococcal infection from a pinprick. Hildegard has failed to respond to any treatments. In desperation, Hildegard's father injects her with prontosil, a red dye with which he has been experimenting in his laboratory. Miraculously, she makes a complete recovery.

This story actually begins three years earlier, when Dr. Domagk observed that prontosil protects mice and rabbits from lethal doses of staphylococci and streptococci. He discovered this by screening thousands of dyes (which are, in actuality, simply chemicals that bind to proteins) for antibacterial activity. When his daughter became ill, however, Domagk was not sure whether prontosil's antibacterial efficacy in mice would carry over to infections in humans. He kept his emergency test of the drug a secret, until data from other physicians came back indicating that the drug had been successful in curing other patients of their infections. In 1939, Gerhard Domagk was awarded the Nobel Prize in Physiology or Medicine for his discovery of prontosil.

## QUESTIONS

1. What is the mechanism responsible for the antibacterial action of prontosil?
2. Why does prontosil kill bacteria but not human cells?
3. What has caused the utility of drugs such as prontosil to decline over the past 65 years?
4. Why are drugs of the same class as prontosil now used in combination with other antibacterial agents?

## BIOCHEMISTRY OF NUCLEOTIDE METABOLISM

The central dogma of molecular biology states that DNA contains all the information necessary to encode cellular macromolecules—specifically, that DNA is transcribed into RNA, and RNA is then translated into proteins. Antimetabolites inhibit the synthesis of nucleotides, which are the building blocks of both DNA and RNA. Figure 31-1A provides an overview of nucleotide synthesis, and Figure 31-1B shows the steps at which the drugs discussed in this chapter inhibit nucleotide metabolism.

### NUCLEOTIDE SYNTHESIS

Nucleotides, the precursors of DNA and RNA, include the **purine** nucleotides and the **pyrimidine** nucleotides. Purines and pyrimidines are the bases that are used to determine the chemical code within DNA and RNA. Adenine and guanine are purines; cytosine, thymine, and uracil are pyrimidines. **Nucleosides** are derivatives of purines and pyrimidines that are conjugated to ribose or deoxyribose. **Nucleotides** are monophosphate, diphosphate, and triphosphate esters of the corresponding nucleosides. For example, an adenine base covalently linked to a ribose sugar and a diphosphate ester is called adenosine diphosphate (ADP). The various purine and pyrimidine bases, nucleosides and nucleotides are shown in Table 31-1.

Nucleotide synthesis involves three general sets of reactions: (1) synthesis of ribonucleotides; (2) reduction of ribonucleotides to deoxyribonucleotides; and (3) conversion of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) (Fig. 31-2). Ribonucleotide synthesis differs for purines and pyrimidines; therefore, the synthesis of each class of molecules is discussed individually. All ribonucleotides are reduced to deoxyribonucleotides by a single enzyme, ribonucleotide reductase. Deoxyribonucleotides generated from ribonucleotides and from dUMP are used for DNA synthesis. Because folate is an essential cofactor for the synthesis of purine ribonucleotides and dTMP, folate metabolism is discussed separately.

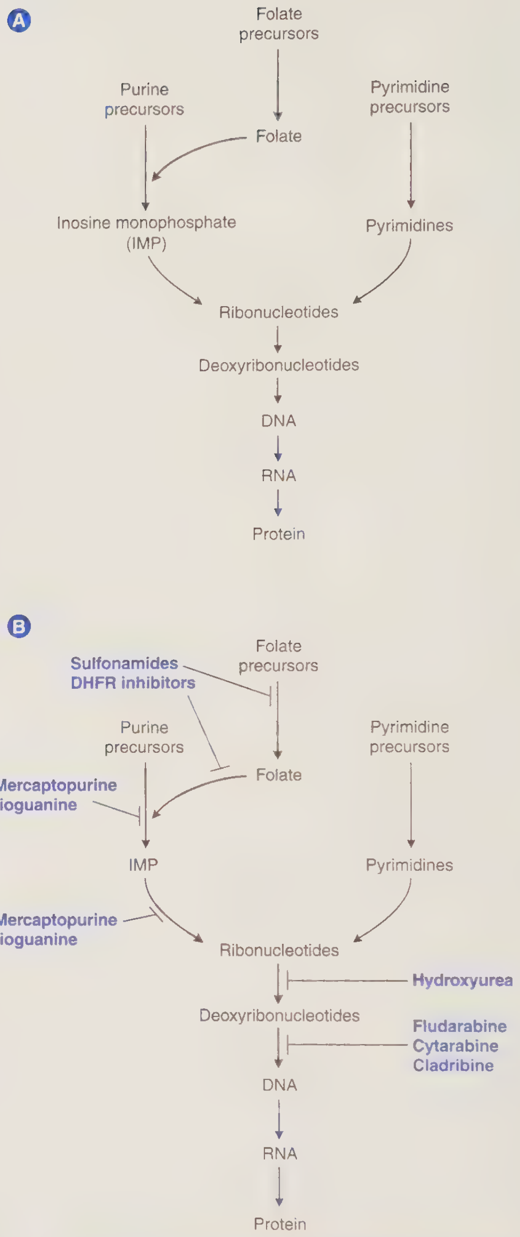
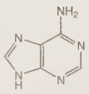
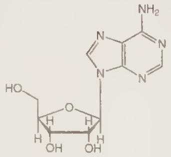
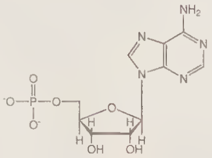
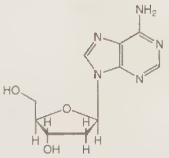
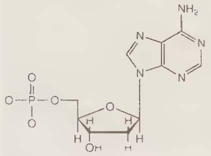
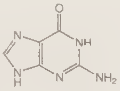
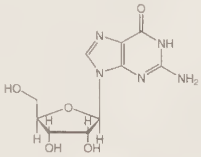
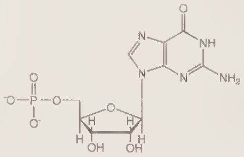
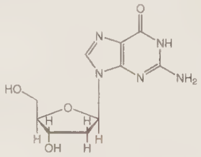
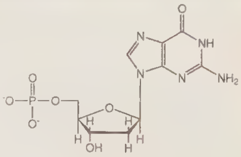
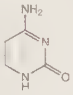
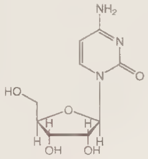
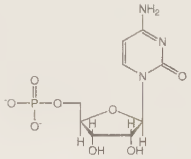
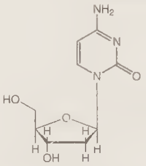
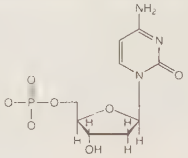
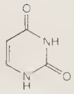
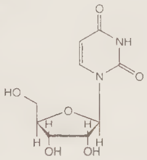
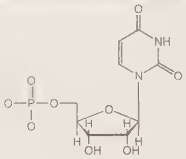
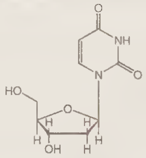
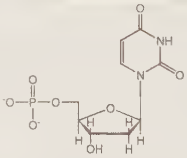
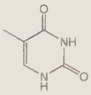
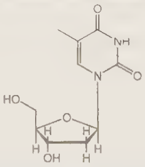
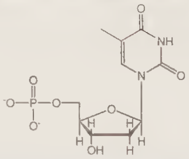

A
Folate precursors
Purine precursors
Pyrimidine precursors
Folate
Inosine monophosphate (IMP)
Pyrimidines
Ribonucleotides
Deoxyribonucleotides
DNA
RNA
Protein
B
Sulfonamides
DHFR inhibitors
Folate precursors
Purine precursors
Pyrimidine precursors
Folate
6-Mercaptopurine
Thioguanine
IMP
Pyrimidines
6-Mercaptopurine
Thioguanine
Ribonucleotides
Hydroxyurea
Deoxyribonucleotides
Fludarabine
Cytarabine
Cladribine
DNA
RNA
Protein

**Figure 31-1. Overview of De Novo Nucleotide Biosynthesis.** **A.** Folate is an essential cofactor in the synthesis of inosine monophosphate (IMP), from which all purine nucleotides are derived. Pyrimidine synthesis does not require folate, although folate is required for the methylation of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) (see Fig. 31-2). Ribonucleotides contain one of the purine or pyrimidine bases linked to ribose phosphate. Subsequent reduction of the ribose at the 2′ position produces deoxyribonucleotides. Deoxyribonucleotides are polymerized into DNA, while ribonucleotides are used to form RNA (not shown). The central dogma of molecular biology states that the DNA code determines the sequence of RNA (transcription), and that RNA is then translated into protein (blue arrows). **B.** Sulfonamides inhibit the synthesis of folate from precursors, while dihydrofolate reductase (DHFR) inhibitors prevent the utilization of folate in purine nucleotide and dTMP synthesis. 6-Mercaptopurine and thioguanine inhibit the formation of purine nucleotides. Hydroxyurea inhibits the enzyme that converts ribonucleotides to deoxyribonucleotides. Fludarabine, cytarabine, and cladribine are purine and pyrimidine analogues that inhibit DNA synthesis. 5-Fluorouracil and flucytosine inhibit the enzyme that converts dUMP to dTMP (not shown).

**TABLE 31-1** Purine and Pyrimidine Derivatives: Bases, Nucleosides, and Nucleotides

| | BASE | RIBONUCLEOSIDE | RIBONUCLEOTIDE | DEOXYRIBONUCLEOSIDE | DEOXYRIBONUCLEOTIDE |
|---|---|---|---|---|---|
| **Purines** |  Adenine (A) |  Adenosine |  Adenylate (AMP) |  Deoxyadenosine |  Deoxyadenylate (dAMP) |
| |  Guanine (G) |  Guanosine |  Guanylate (GMP) |  Deoxyguanosine |  Deoxyguanylate (dGMP) |
| **Pyrimidines** |  Cytosine (C) |  Cytidine |  Cytidylate (CMP) |  Deoxycytidine |  Deoxycytidylate (dCMP) |
| |  Uracil (U) |  Uridine |  Uridylate (UMP) |  Deoxyuridine |  Deoxyuridylate (dUMP) |
| |  Thymine (T) | NONE | NONE |  Deoxythymidine |  Deoxythymidylate (dTMP) |

## Purine Ribonucleotide Synthesis

**Adenine** and **guanine,** the purine bases shown in Table 31-1, are synthesized as components of ribonucleotides (for RNA synthesis) and deoxyribonucleotides (for DNA synthesis) Derivatives of adenine and guanine include ATP, GTP, cAMP, and cGMP, which are also used for energy storage and cell signaling. Purine synthesis begins with the assembly of **inosinate** (IMP) from a ribose phosphate, moieties derived from the amino acids glycine, aspartate, and glutamine, and 1-carbon transfers catalyzed by **tetrahydrofolate** (THF), as shown in Figure 31-2. Because of the central role of THF in purine synthesis, one important chemotherapeutic strategy is to reduce the amount of THF available to the cell and, thereby, to reduce purine synthesis (see below).

Figure 31-3 shows the central role of IMP in purine synthesis. IMP can be aminated to AMP or oxidized to GMP. In turn, AMP and GMP can be converted to ATP and GTP, respectively, and then incorporated into RNA, or reduced to dAMP and dGMP, respectively, as described below.

Purine bases, nucleosides, and nucleotides are readily interconverted by multiple enzymes within the cell. In one such reaction, the enzyme adenosine deaminase (ADA) catalyzes the irreversible conversion of adenosine or 2′-deoxyadenosine to inosine or 2′-deoxyinosine, respectively. Inhibition of ADA causes the intracellular stores of adenosine and 2′-deoxyadenosine to exceed those of the other purines, ultimately resulting in metabolic effects that are toxic to the cell (see the discussion of pentostatin, below).

## Pyrimidine Ribonucleotide Synthesis

Pyrimidine ribonucleotides are synthesized according to the metabolic pathway shown in Figure 31-4. The basic pyrimidine ring, orotate, is assembled from carbamoyl phosphate and aspartate. Orotate then reacts with a ribose phosphate; the

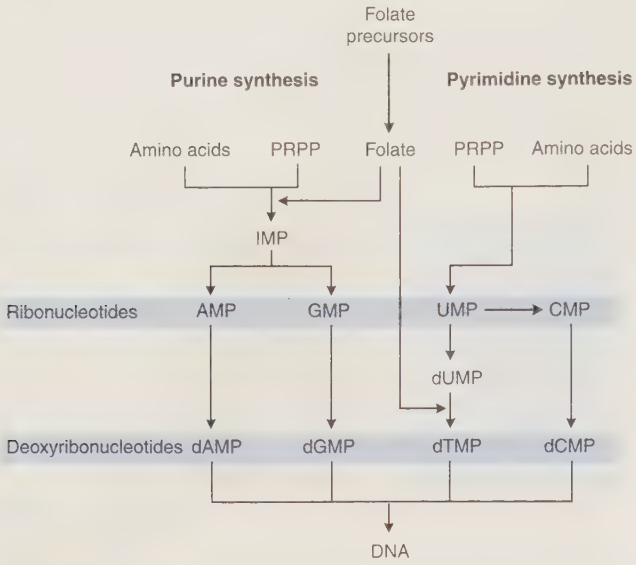


**Figure 31-2. Nucleotide Synthesis.** Purine synthesis (left) begins with the formation of inosine monophosphate (IMP) from amino acids, phoshoribosylpyrophosphate (PRPP), and folate. IMP is aminated to adenylate (AMP) or oxidized to guanylate (GMP). The ribonucleotides AMP and GMP are reduced to form the deoxyribonucleotides deoxyadenosine monophosphate (dAMP) and deoxyguanosine monophosphate (dGMP), respectively. (The conversion of ribonucleotides to deoxyribonucleotides actually takes place at the level of the corresponding diphosphates and triphosphates, e.g., ADP → dADP and ATP → dATP.) Pyrimidine synthesis (right) begins with the formation of orotate from aspartate and carbamoyl phosphate (not shown). Orotate is ribosylated and decarboxylated to uridylate (UMP); amination of UMP yields cytidylate (CMP). (The conversion of UMP to CMP actually takes place at the level of the corresponding triphosphates, i.e., UTP → CTP.) The ribonucleotides UMP and CMP are reduced to form the deoxyribonucleotides deoxyuridine monophosphate (dUMP) and deoxycytidine monophosphate (dCMP). dUMP is converted to deoxythymidine monophosphate (dTMP) in a reaction that depends on folate. At the level of the corresponding triphosphates (not shown), deoxyribonucleotides are incorporated into DNA and ribonucleotides are incorporated into RNA (not shown). Note the central role of folate as an essential cofactor in the synthesis of purine nucleotides and dTMP.

decarboxylation product of this reaction yields **uridylate** (UMP). As with IMP in purine synthesis, UMP has a central role in pyrimidine synthesis. UMP is itself a nucleotide component of RNA, as well as the common precursor of the RNA and DNA components cytidylate (CMP), deoxycytidylate (dCMP) and deoxythymidylate (dTMP). CTP is formed by the amination of UTP.

## Ribonucleotide Reduction and Thymidylate Synthesis

Ribonucleotide synthesis is required for RNA synthesis because, in RNA synthesis, the ribonucleotides ATP, GTP, UTP, and CTP are assembled on a DNA template into RNA. Alternatively, ribonucleotides can be reduced at the 2′ position on ribose to form the deoxyribonucleotides dATP, dGTP, dUTP and dCTP. The conversion of ribonucleotides to deoxyribonucleotides is catalyzed by the enzyme **ribonucleotide reductase.** (In actuality, ribonucleotide reductase uses as substrates the diphosphate forms of the four ribonucleotides to produce dADP, dGDP, dUDP, and dCDP; nucleotides can, however, be readily interconverted among their monophosphate, diphosphate and triphosphate forms.)

Note, in Figures 31-2 through 31-4, that ribonucleotide reductase catalyzes the formation of the DNA precursors dATP, dGTP, and dCTP. The DNA precursor dTTP, however, is not synthesized directly by ribonucleotide reductase. Rather, dUMP must be modified to form dTMP. As can be seen in Table 31-1, dTMP is the product of dUMP methylation. The methylation of dUMP to dTMP is catalyzed by **thymidylate synthase,** with methylenetetrahydrofolate (MTHF) serving as the donor of the methyl group (Fig. 31-4). As MTHF donates its methyl group, it is oxidized to dihydrofolate (DHF). DHF must be reduced to THF by **dihydrofolate reductase** (DHFR) and then converted to MTHF to serve as the cofactor for another cycle of dTMP synthesis. Inhibition of DHFR prevents the regeneration of tetrahydrofolate and, thereby, inhibits the conversion of dUMP to dTMP, eventually resulting in an insufficient cellular level of dTMP for DNA replication.

## Folate Metabolism

**Folic acid** is a vitamin that participates in a number of enzymatic reactions involving the transfer of one-carbon units. These reactions are essential for the biosynthesis of DNA and RNA precursors, the amino acids glycine, methionine, and glutamic acid, the formyl-methionine initiator tRNA, and other essential metabolites. Given the importance of folate metabolism in the biochemistry of the cell, it is not surprising that inhibition of folate biosynthesis and interference with the folate cycle have been used widely in the treatment of bacterial infections, parasitic infections, and cancer.

The structure of folic acid contains three chemical moieties (Fig. 31-5): a pteridine ring system, para-aminobenzoic acid (PABA), and the amino acid glutamate. (Because of its ability to absorb UV light, PABA is the active ingredient in many topical sunscreens.) Folate is an essential vitamin for humans that must be provided intact in the diet. In lower organisms, however, folate is synthesized from precursors, as shown in Figure 31-6.

Both dietary folate and folate synthesized from precursors enter the folate cycle (Fig. 31-6). In this cycle, dihydrofolate is reduced to tetrahydrofolate by dihydrofolate reductase (DHFR). Tetrahydrofolate then enters the many metabolic interconversions that involve one-carbon transfers. For example, congeners of tetrahydrofolate are essential donors of carbon atoms in the synthesis of IMP (leading to AMP and GMP) and in the conversion of dUMP to dTMP (Fig. 31-2). In all of these reactions, tetrahydrofolate congeners donate a carbon atom and, in the process, are oxidized to dihydrofolate. For further rounds of nucleotide synthesis to occur, the dihydrofolate must be reduced to tetrahydrofolate by DHFR.

## NUCLEIC ACID SYNTHESIS

Provided that sufficient levels of nucleotides are available, DNA and RNA can be synthesized, and protein synthesis,

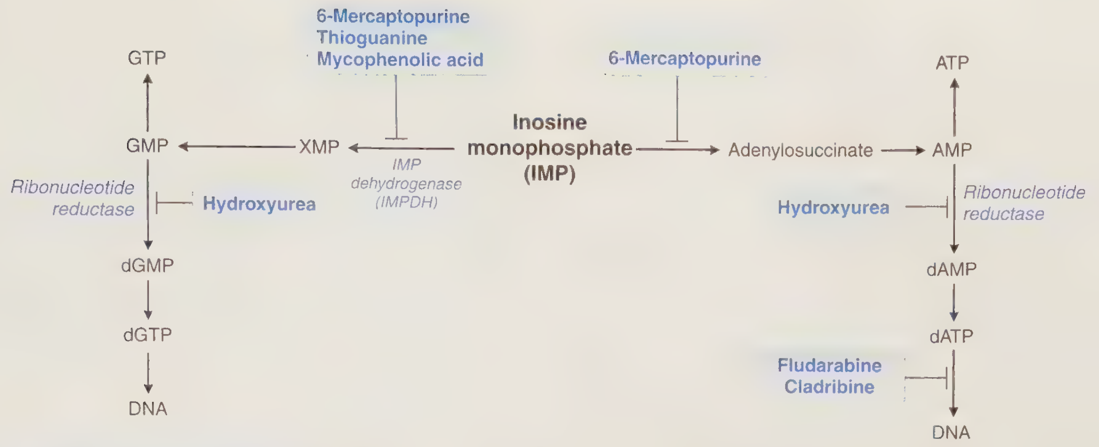


**Figure 31-3. Details of Purine Synthesis.** Inosinate, or inosine monophosphate (IMP), occupies a central position in the synthesis of purine nucleotides. IMP is oxidized by IMP dehydrogenase (IMPDH) to xanthylate (XMP), which is converted to guanosine monophosphate (GMP). GMP can be incorporated into DNA or RNA as deoxyguanosine triphosphate (dGTP) or guanosine triphosphate (GTP), respectively. Alternatively, IMP can be aminated to adenosine monophosphate (AMP) through an adenylosuccinate intermediate. AMP can be incorporated into DNA or RNA as deoxyadenosine triphosphate (dATP) or adenosine triphosphate (ATP), respectively. 6-Mercaptopurine, thioguanine, and mycophenolic acid inhibit IMPDH, and thus interrupt GMP synthesis. 6-Mercaptopurine also inhibits the conversion of IMP to adenylosuccinate and, thus, interrupts AMP synthesis. Hydroxyurea inhibits ribonucleotide reductase and, thus, inhibits formation of the deoxyribonucleotides required for DNA synthesis. Fludarabine and cladribine are halogenated adenosine analogues that inhibit DNA synthesis.

cell growth, and cell division can occur. Many drugs, including the antimetabolites discussed below, can inhibit both DNA and RNA synthesis. To avoid repetition, a detailed discussion of DNA synthesis is given in Chapter 32, and a discussion of RNA synthesis is given in Chapter 33. For the purposes of this chapter, the reader should be aware that *RNA and DNA are formed by polymerization of ribonucleotides and deoxyribonucleotides, respectively.* RNA polymers are elongated by the enzyme **RNA polymerase**, and DNA is elongated by **DNA polymerase**. Although antimetabolites inhibit primarily the enzymes that mediate nucleotide synthesis, some antimetabolites also inhibit DNA and RNA polymerases (see below).

## PHARMACOLOGIC CLASSES AND AGENTS

Antimetabolites can be divided into inhibitors of folate metabolism, inhibitors of purine metabolism, inhibitors of ribonucleotide reductase, and nucleotide analogues that are incorporated into DNA. Throughout the following discussion of antimetabolite pharmacology, it is helpful to keep in mind the basis for the selectivity of each individual agent. As outlined in Chapter 30, the basis for drug selectivity in antimicrobial and antineoplastic pharmacology is the relative uniqueness of the drug target. Thus, selectivity can take the form of: (1) a genetic or biochemical pathway that is unique to the pathogen or cancer cell; (2) a structure (isoform) of a protein that is unique to the pathogen or cancer cell; or (3) a metabolic requirement that is unique to the pathogen or cancer cell. Where relevant, the following discussion emphasizes the basis for selectivity of each therapeutic agent.

## INHIBITORS OF FOLATE METABOLISM

Inhibitors of folate metabolism include inhibitors of dihydropteroate synthase and inhibitors of dihydrofolate reductase. In each case, drugs that structurally resemble the physiologic substrate of the enzyme act as enzyme inhibitors.

### Inhibitors of Dihydropteroate Synthase

Bacteria are unable to take up folic acid from the environment and, therefore, must synthesize the vitamin de novo from para-aminobenzoic acid (PABA), pteridine, and glutamate (Fig. 31-6). Mammalian cells, in contrast, use folate receptors and folate carriers in the plasma membrane to scavenge the intact vitamin. This fundamental metabolic difference between pathogen and host cells makes dihydropteroate synthase an ideal target for antibacterial therapy. The **sulfa** drugs, such as **sulfamethoxazole** and **sulfadiazine,** are PABA analogues that competitively inhibit dihydropteroate synthase and, thereby, prevent the synthesis of folic acid in bacteria. The lack of folic acid, in turn, prevents bacterial synthesis of purines, pyrimidines, and some amino acids, and eventually results in cessation of bacterial growth. Sulfa drugs are bacteriostatic because they prevent bacterial growth but do not kill the bacteria. There are two structural classes of sulfa drugs: sulfonamides and sulfones.

#### *Sulfonamides*

As demonstrated in the case of Hildegard Domagk, **sulfonamides** were the first modern agents to be employed in the treatment of bacterial infections (prontosil is a sulfonamide precursor). Figure 31-5 shows the similarity in structure between PABA and the sulfonamide analogues **sulfanilamide**,

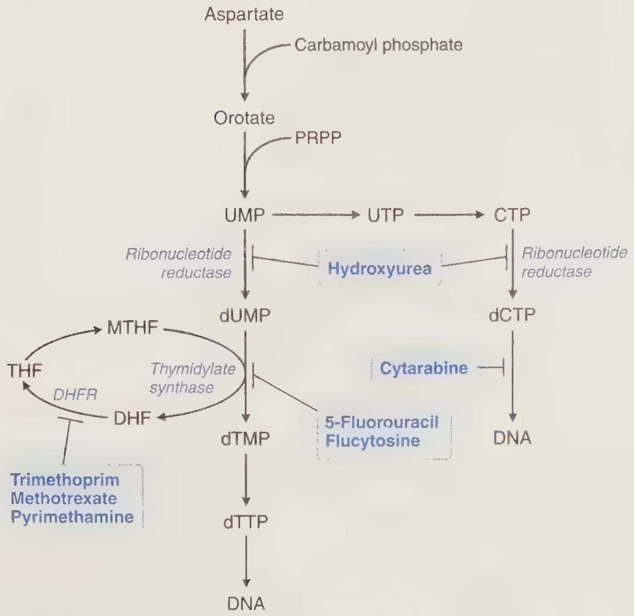
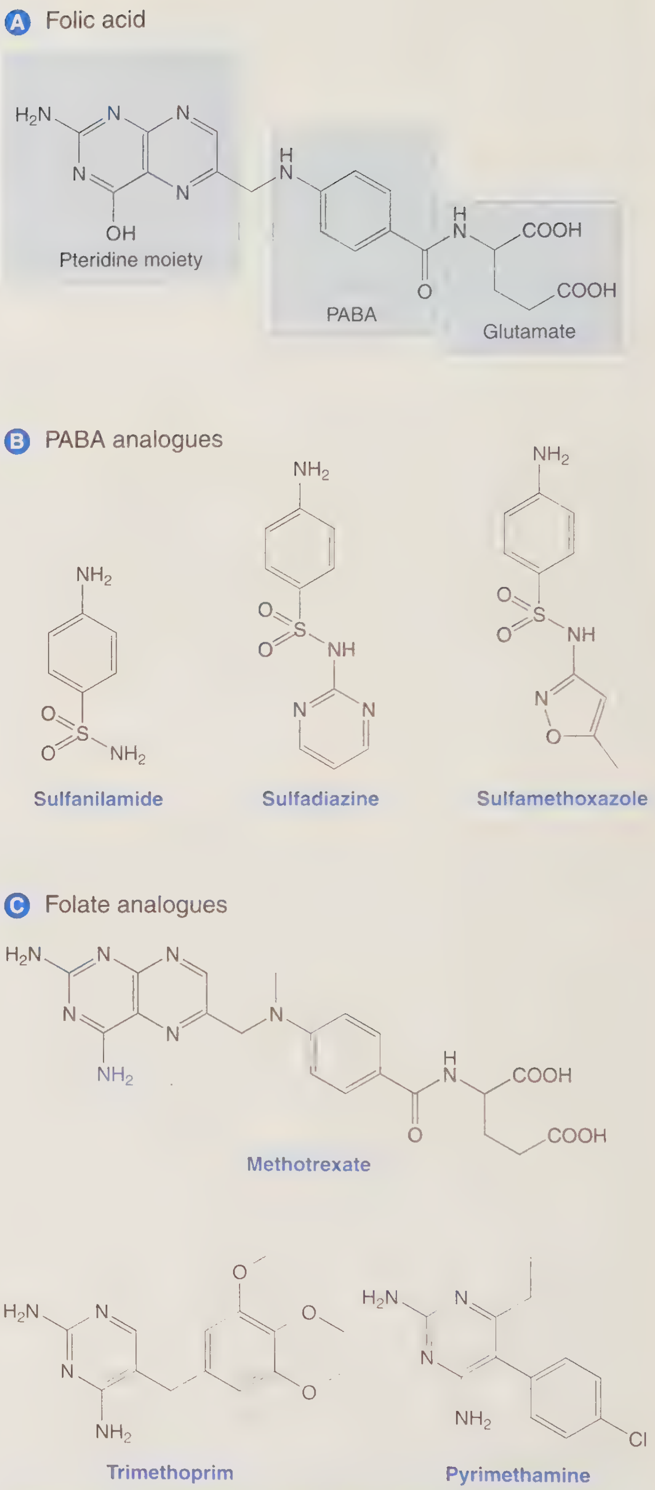

Aspartate
Carbamoyl phosphate
Orotate
PRPP
UMP
UTP
CTP
Ribonucleotide reductase
Hydroxyurea
Ribonucleotide reductase
dUMP
dCTP
MTHF
THF
DHFR
Thymidylate synthase
Cytarabine
DHF
5-Fluorouracil
Flucytosine
dTMP
DNA
Trimethoprim
Methotrexate
Pyrimethamine
dTTP
DNA

**Figure 31-4. Details of Pyrimidine Synthesis.** Aspartate (an amino acid) and carbamoyl phosphate combine to form orotate, which then combines with phosphoribosylpyrophosphate (PRPP) to form uridylate (UMP). UMP occupies a central position in the synthesis of pyrimidine nucleotides. UMP can be sequentially phosphorylated to uridine triphosphate (UTP). UTP is incorporated into RNA (not shown) or aminated to form cytidine triphosphate (CTP). CTP is incorporated into RNA (not shown) or reduced by ribonucleotide reductase to deoxycytidine triphosphate (dCTP), which is incorporated into DNA. Alternatively, UMP can be reduced to deoxyuridylate (dUMP). Thymidylate synthase converts dUMP to deoxythymidylate (dTMP), in a reaction that depends on folate. dTMP is phosphorylated to deoxythymidine triphosphate (dTTP), which is incorporated into DNA. Hydroxyurea inhibits the formation of deoxyribonucleotides and, thereby, inhibits DNA synthesis. Cytarabine, a cytidine analogue, inhibits the incorporation of dCTP into DNA. 5-Fluorouracil and flucytosine inhibit dTMP synthesis by inhibiting thymidylate synthase. Trimethoprim, methotrexate, and pyrimethamine inhibit dihydrofolate reductase (DHFR), the enzyme responsible for regenerating tetrahydrofolate (THF) from DHF. By inhibiting DHF reductase, these drugs inhibit the formation of methylenetetrahydrofolate (MTHF), which is the folate compound that is required for dTMP synthesis.

**sulfadiazine**, and **sulfamethoxazole**. Sulfonamides are highly selective drugs, because bacterial growth requires activity of the enzyme that is inhibited by the sulfonamides, whereas mammalian cells do not even express this enzyme. Therefore, mammalian cells are essentially unaffected by the sulfonamides, and the use of these drugs is relatively free of side effects (except in the special case of neonates, noted below).

Despite the exquisite selectivity of the sulfonamides, the development of resistance to these drugs has resulted in their diminished use. Resistance to sulfonamides can develop because of: (1) overproduction of the endogenous substrate, PABA, by bacteria exposed to sulfonamides; (2) a mutation in the PABA binding site on dihydropteroate synthase, resulting in reduced affinity of the enzyme for sulfonamides; or (3) decreased permeability of the bacterial membrane to sulfonamides. Some resistant streptococci produce levels of


A Folic acid

B PABA analogues

C Folate analogues

H₂N
N
OH
Pteridine moiety
H N
PABA
COOH
Glutamate
NH₂
NH
Sulfanilamide
Sulfadiazine
Sulfamethoxazole
Methotrexate
Trimethoprim
Pyrimethamine
Cl

**Figure 31-5. Structures of Folic Acid, PABA Analogues (Sulfonamides), and Folate Analogues (Dihydrofolate Reductase Inhibitors).** **A.** Folic acid is formed by the condensation of pteridine, para-aminobenzoic acid (PABA), and glutamate (see Fig. 31-6). Folate is the deprotonated form of folic acid. **B.** PABA analogues (sulfonamides) structurally resemble PABA. These drugs inhibit dihydropteroate synthase, the enzyme that catalyzes the formation of dihydropteroic acid from PABA and pteridine (see Fig. 31-6). **C.** Folate analogues (dihydrofolate reductase inhibitors) structurally resemble folic acid. These drugs inhibit dihydrofolate reductase, the enzyme that converts dihydrofolate to tetrahydrofolate.

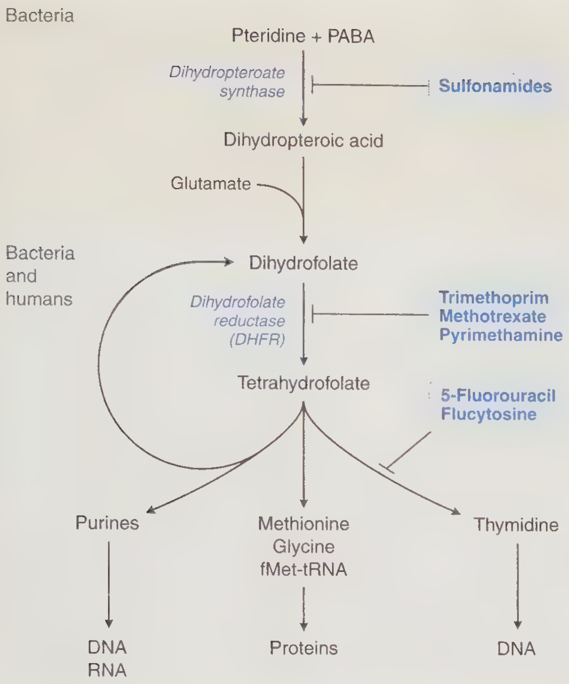


**Figure 31-6. Folate Synthesis and Functions.** Folate synthesis begins with the formation of dihydropteroic acid from pteridine and para-aminobenzoic acid (PABA); this reaction is catalyzed by dihydropteroate synthase. Glutamate and dihydropteroic acid condense to form dihydrofolate (DHF). DHF is reduced to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR). THF and its congeners (not shown) serve as 1-carbon donors in numerous reactions necessary for the formation of DNA, RNA, and proteins. In each such reaction, the reduced folate (here, shown as THF) becomes oxidized to DHF, and the THF must then be regenerated via reduction by DHFR. Inhibitors of folate metabolism target three steps in the folate pathway. Sulfonamides inhibit dihydropteroate synthase; trimethoprim, methotrexate, and pyrimethamine inhibit DHFR; 5-fluorouracil (5-FU) and flucytosine inhibit thymidylate synthase (see Fig. 31-4). Note that bacteria synthesize folate de novo from pteridine and PABA, while humans require dietary folate.

PABA that are 70-fold greater than the normal value. Decreased membrane permeability to sulfonamides can be conferred by bacterial transfer of a resistance plasmid.

Because of the high incidence of sulfonamide resistance in the bacterial population, these drugs are rarely administered as single agents. Instead, they are commonly administered in conjunction with a synergistic drug such as trimethoprim or pyrimethamine, as discussed below.

Sulfonamides compete with bilirubin for binding sites on serum albumin, and can cause kernicterus in newborns. **Kernicterus,** the condition characterized by markedly elevated concentrations of unconjugated (free) bilirubin in the blood of neonates, can lead to severe brain damage. For this reason, newborns should not be treated with sulfonamides.

### *Sulfones*

Used in the treatment of leprosy and *Pneumocystis carinii* pneumonia, **dapsone** is a member of the sulfone class of dihydropteroate synthase inhibitors. Because the mechanism of action of dapsone is the same as that of the sulfonamides, dapsone and trimethoprim or pyrimethamine can also be used as a synergistic drug combination (see below). Interestingly, one relatively common side effect of dapsone is that about 5% of patients develop **methemoglobinemia** following administration of the drug. Susceptible patients are typically deficient in the erythrocyte enzyme glucose-6-phosphate dehydrogenase, which is involved in the detoxification of endogenous and exogenous oxidizing agents (such as dapsone).

## Inhibitors of Dihydrofolate Reductase

Dihydrofolate reductase (DHFR) is the enzyme that reduces dihydrofolate (DHF) to tetrahydrofolate (THF). Several drugs, including trimethoprim, pyrimethamine, and methotrexate, are folate analogues that competitively inhibit DHFR and prevent the regeneration of THF from DHF (Fig. 31-5C). By doing so, these drugs prevent the synthesis of purine nucleotides as well as the methylation of dUMP to dTMP (see above). Pharmacologic inhibition of DHFR is used both in the treatment of infection and in cancer chemotherapy.

Many inhibitors of DHFR have been developed. As shown in Table 31-2, **methotrexate** is a potent (subnanomolar) inhibitor of DHFR, although it exhibits little selectivity among the mammalian, bacterial, and protozoal isoforms of the enzyme. In contrast, inhibitors with structures that are more divergent from that of folate, such as **trimethoprim** and **pyrimethamine** (see Fig. 31-5), show considerable selectivity of DHFR inhibition among the various isoforms of the enzyme. Thus, trimethoprim is a potent and selective antibacterial agent, whereas pyrimethamine is a potent and selective antimalarial drug.

Why are trimethoprim and pyrimethamine each selective for one particular isoform of DHFR, while methotrexate is not? The amino acid sequences of DHFR enzymes from many species have been determined, and these sequences vary greatly among the bacteria, protozoans, and humans. In contrast, the substrates of DHFR, dihydrofolate and NADPH, have not changed at all over the course of evolution. Nonetheless, all of the various enzyme isoforms can efficiently catalyze the reduction of DHF to THF. (Similar observations have been made for many enzymes, including glycolytic enzymes.) This implies that there are many ways to encode a protein that

**TABLE 31-2** $IC_{50}$ Values for Three Dihydrofolate Reductase Inhibitors

| | DHFR ISOFORM | | |
|---|---|---|---|
| **DHFR INHIBITOR** | ***E. Coli*** **DHFR** | **Malarial DHFR** | **Mammalian DHFR** |
| Trimethoprim | **7** | 1,800 | 350,000 |
| Pyrimethamine | 2,500 | **0.5** | 1,800 |
| Methotrexate | 0.1 | 0.7 | **0.2** |

DHFR, dihydrofolate reductase. $IC_{50}$, concentration required for 50% enzyme inhibition. All values are reported in nM ($10^{-9}$ M) units. Trimethoprim and pyrimethamine are selective inhibitors of the *E. coli* and malarial isoforms of DHFR, respectively. In contrast, methotrexate is a nonselective inhibitor of all three DHFR isoforms.

### BOX 31-1. The Future of Dihydrofolate Reductase Inhibitors

Despite the effectiveness of the available DHFR inhibitors, there is much interest in developing new compounds. Drug resistance is a problem, and for many species, no selective inhibitor is available. Recent developments have allowed researchers to determine the amino acid sequences and three-dimensional structures of DHFRs from several different organisms, including complexes of the enzyme with small-molecule inhibitors. These studies have provided a molecular basis for understanding both enzymatic catalysis and inhibition (e.g., why inhibitors, such as trimethoprim, effectively inhibit bacterial but not mammalian DHFRs). Importantly, these studies also suggest how one could design a new drug that is capable of even more potent or selective inhibition. Thus, instead of employing "random screening" methods (which, in fact, have been successful in the development of many current enzyme inhibitors), it may soon be possible to apply this powerful technology for efficient and rational design of more selective agents. "Rational drug design" is currently in its infancy, but this technology holds substantial promise for the future.

contains the various binding sites and conformational flexibility required for catalysis. The basis for selectivity must, therefore, reside in differences in enzyme structure that are largely irrelevant for binding of the natural substrates, but that have an important role in analogue (drug) binding. Correspondingly, the very close structural resemblance between methotrexate (MTX) and the normal substrate dihydrofolate (see Fig. 31-5) may explain why MTX exhibits little selectivity for DHFR isoforms across a wide range of species, whereas the more divergent structures of trimethoprim and pyrimethamine are associated with higher selectivity of isoform binding and inhibition. Increased understanding of the molecular basis for DHFR inhibition may lead to the development of more selective agents (Box 31-1).

### Methotrexate

As described above, **methotrexate (MTX)** is a folate analogue that reversibly inhibits DHFR. In mammalian cells, DHFR inhibition causes a critical shortage of intracellular supplies of tetrahydrofolate, resulting in cessation of de novo purine and thymidylate synthesis and, therefore, cessation of DNA and RNA synthesis. Because the synthesis of DNA is halted, mammalian cells treated with methotrexate are arrested in the S phase of the cell cycle (see Chapter 30).

The basis for the relative selectivity of methotrexate for cancer cells compared to normal cells is thought to be that rapidly growing cancer cells have an increased requirement for the various compounds that depend on folate intermediates, especially those (such as purines and thymidylate) required for DNA synthesis. In addition, malignant cells may be more susceptible than normal cells to the apoptosis-inducing effects of MTX (see below). The use of high-dose MTX in cancer chemotherapy has been broadened by the application of **folinic acid rescue.** In this technique, folinic acid (N-5 formyltetrahydrofolate, also called **leukovorin**) is administered to the patient several hours after an otherwise lethal dose of methotrexate. The rationale for this technique is that the malignant cells are killed selectively, while the normal cells are "rescued" by the folinic acid. The molecular explanation for the effectiveness of folinic acid rescue is unclear. One hypothesis suggests that normal (nonmalignant) cells are able to concentrate the folinic acid (and, thus, to protect themselves from the effects of MTX), whereas malignant cells have a reduced rate of folinic acid transport (and, therefore, are preferentially harmed by high doses of MTX). Another hypothesis suggests that high-dose MTX induces apoptosis in malignant cells but cell cycle arrest in normal cells; the normal cells are then able to use the folinic acid to resume cell growth and division, while the malignant cells are already committed to programmed cell death (see the discussion of p53 and apoptosis in Chapter 30).

MTX is used to treat many tumor types, including carcinomas of the breast, lung, head and neck, acute lymphoblastic leukemia, and choriocarcinoma. MTX can also be used to treat psoriasis and to treat early stage ectopic pregnancy. Methotrexate toxicity is manifested primarily in rapidly dividing host cells, causing damage to the gastrointestinal mucosa and the bone marrow. These effects are generally reversible after therapy is discontinued. MTX is extremely toxic to the fetus, because folic acid is essential for the proper differentiation of fetal cells and for closure of the neural tube. MTX has recently undergone clinical trials as an abortion-inducing agent, either alone or in combination with the prostaglandin analogue misoprostol.

### Trimethoprim

**Trimethoprim** is a folate analogue that selectively inhibits bacterial DHFR (Fig. 31-5C; Table 31-2) and, thereby, prevents the conversion of DHF to THF. As with the sulfonamides, trimethoprim is bacteriostatic. Because trimethoprim is excreted unchanged in the urine, it can be used as a single agent to treat uncomplicated urinary tract infections. For most infections, however, trimethoprim is used in combination with sulfamethoxazole. The rationale for this combination antibacterial chemotherapy is described below.

### Pyrimethamine

**Pyrimethamine** is a folate analogue that selectively inhibits parasitic DHFR (Fig. 31-5C; Table 31-2). Pyrimethamine is currently the only effective chemotherapeutic agent against toxoplasmosis; for this indication, it is typically administered in combination with sulfadiazine. Pyrimethamine has also been used to treat malaria, although widespread resistance has limited its effectiveness in recent years. Further discussion of the therapeutic applications of pyrimethamine and sulfadiazine can be found in Chapter 37, Pharmacology of Parasitic Infections.

### Synergy of DHFR Inhibitors and Sulfonamides

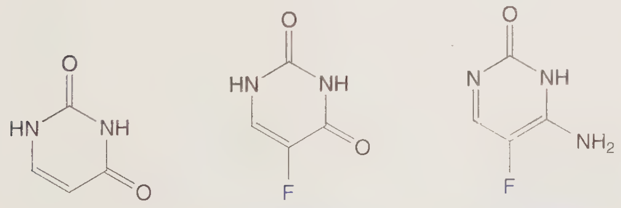
Both trimethoprim and pyrimethamine can be used in combination with sulfonamides to block sequential steps in the biosynthetic pathway leading to tetrahydrofolate (Fig. 31-6).

This type of combination chemotherapy, called **sequential blockade,** has been very effective in the treatment of parasitic infections (pyrimethamine and sulfadiazine) and bacterial infections (trimethoprim and sulfamethoxazole). One rationale for the use of a DHFR inhibitor and a sulfa drug in combination is the marked synergistic interaction between these two classes of drugs (see Chapter 38, Principles of Combination Chemotherapy). The sulfonamide decreases the intracellular concentration of dihydrofolate; this increases the effectiveness of the DHFR inhibitor, which competes with dihydrofolate for binding to the enzyme. The sulfa/DHFR inhibitor combination can also be effective in treating strains of bacteria and parasites that exhibit resistance to monotherapy with a DHFR inhibitor. Typically, this drug resistance phenotype is caused by the expression of a structurally altered DHFR that has a lower affinity for the inhibitor. The tradeoff for the bacteria or parasite is that the altered DHFR also has a lower affinity for the natural ligand dihydrofolate. In such strains, sulfonamide treatment can decrease the intracellular concentration of dihydrofolate to the point that the altered DHFR cannot meet the metabolic requirements of the cell.

Another important rationale for the use of a combination such as trimethoprim/sulfamethoxazole is that resistance to trimethoprim alone or sulfamethoxazole alone develops rather quickly, whereas resistance to the drug combination develops much more slowly. Because the two drugs act on different enzymes, two different mutations would have to occur simultaneously for the bacteria to develop resistance to the drug combination. Compared to the rate of development of a single mutation, the likelihood that two mutations will occur simultaneously is much lower (see Chapter 38).

## INHIBITORS OF THYMIDYLATE SYNTHASE

As described above, thymidylate (dTMP) is synthesized by the methylation of 2′-deoxyuridylate (dUMP). This reaction, which is catalyzed by thymidylate synthase, requires methylenetetrahydrofolate (MTHF) as a cofactor (Fig. 31-4). **5-Fluorouracil** (5-FU; Fig. 31-7) inhibits DNA synthesis, primarily by interfering with the biosynthesis of thymidylate. 5-FU is first converted to 5-fluoro-2′-deoxyuridylate (FdUMP) by the same pathways that convert uracil to dUMP. FdUMP then inhibits **thymidylate synthase** by forming, together with MTHF, a stable, covalent ternary enzyme–substrate–cofactor complex. Cells deprived of dTMP for a sufficient period of time undergo so-called "thymineless death." 5-FU can also be metabolized to floxuridine triphosphate (FUTP), which can be incorporated into mRNA in place of uridylate and can, thereby, interfere with RNA processing. Either inhibition of thymidylate synthase by FdUMP, or interference with RNA processing by FUTP, or a combination of the two mechanisms could explain the toxic effect of 5-FU on cells. Recent evidence, however, demonstrates that certain 5-FU congeners, which inhibit thymidylate synthase but are not incorporated into RNA, show antitumor efficacy similar to that of 5-FU. This finding points to thymidylate synthase inhibition as the dominant mechanism of 5-FU activity.



Uracil

**5-Fluorouracil (5-FU)**

**Flucytosine**

**Figure 31-7. Structures of Uracil, 5-Fluorouracil, and Flucytosine.** Note the structural similarity among uracil, 5-fluorouracil (5-FU), and flucytosine. Uracil is the base in the endogenous substrate for thymidylate synthase (see Fig. 31-4). 5-FU is an irreversible inhibitor of thymidylate synthase. Cytosine deaminase (expressed by fungi and some gastrointestinal flora) converts flucytosine to 5-FU. The selective expression of cytosine deaminase by fungi makes flucytosine a selective antifungal agent.

5-FU is used as an antineoplastic agent, especially in the treatment of carcinomas of the breast and gastrointestinal tract. 5-FU has also been used in the topical treatment of premalignant keratoses of the skin and of multiple superficial basal-cell carcinomas. Because 5-FU depletes thymidylate from normal cells as well as cancer cells, this agent is highly toxic and must be used with care.

Elucidation of the mechanism of action of 5-FU has led to the use of folinic acid (leukovorin) in combination with 5-FU as front-line chemotherapy for colorectal cancer. Because 5-FU inhibits thymidylate synthase by forming a ternary complex involving the enzyme (thymidylate synthase), substrate (5-FdUMP), and cofactor (methylenetetrahydrofolate (MTHF)), it was hypothesized that increasing the levels of MTHF would potentiate the activity of 5-FU. Clinical trials proved this hypothesis to be correct, by showing that the efficacy of the combined regimen is higher than that of 5-FU alone. This is an important example of the use of mechanistic knowledge to improve the clinical effectiveness of existing drugs.

**Flucytosine (5-FC)** is a fluorinated cytosine analogue (Fig. 31-7) that is used clinically as an antifungal agent. All fungi that are sensitive to flucytosine express **cytosine deaminase,** an enzyme that converts 5-FC to 5-FU. Because mammalian cells do not catalyze this reaction, flucytosine is a highly selective antifungal drug. Within the fungal cell, 5-FU inhibits DNA and RNA synthesis, as described above. The moderate toxicity associated with flucytosine use may be related to conversion of the drug to 5-FU by intestinal flora, or to release of 5-FU by fungi.

## INHIBITORS OF PURINE METABOLISM

**6-Mercaptopurine** (6-MP) and **azathioprine** (AZA), a prodrug that is nonenzymatically converted to 6-MP in tissues, are inosine analogues that inhibit interconversions among purine nucleotides (Fig. 31-8). 6-Mercaptopurine contains a sulfur atom in place of the native keto group at C-6 of the purine ring (Fig. 31-8). After its entry into cells, mercaptopurine is converted by the enzyme **hypoxanthine-guanine phosphoribosyl transferase** (HGPRT, see Chapter 46) to the nucleotide form, 6-thioinosine-5′-monophosphate (T-IMP). T-IMP is thought to inhibit purine nucleotide synthesis by several mechanisms. First, T-IMP inhibits the enzymes that convert IMP to AMP and GMP, including inosine monophosphate dehydrogenase (IMPDH) (Fig. 31-3). Second, T-IMP (as with AMP and GMP) is a "feedback" inhibitor

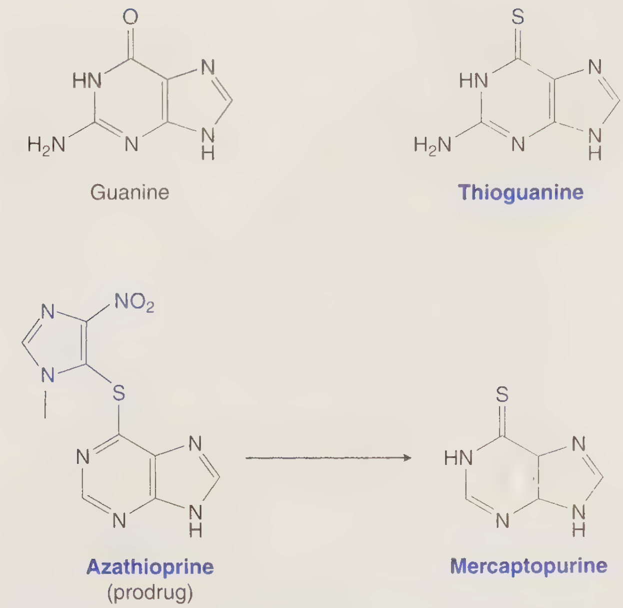


**Figure 31-8. Structures of Guanine, Thioguanine, Azathioprine, and Mercaptopurine.** Thioguanine, azathioprine, and mercaptopurine are structural analogues of purines. Thioguanine resembles guanine, and can be ribosylated and phosphorylated in parallel with endogenous nucleotides. The nucleotide forms of thioguanine irreversibly inhibit IMPDH (see Fig. 31-3) and, upon incorporation into DNA, inhibit DNA replication. Azathioprine is a prodrug form of mercaptopurine; azathioprine reacts with sulfhydryl compounds in the liver (e.g., glutathione) to release mercaptopurine. The nucleotide form of mercaptopurine, thioinosine monophosphate (T-IMP), inhibits the enzymes that convert IMP to AMP and GMP (see Fig. 31-3). T-IMP also inhibits the first committed step in purine nucleotide synthesis.

of the enzyme that synthesizes phosphoribosylamine, which is the first committed step in purine nucleotide synthesis. Both of these mechanisms lead to marked decreases in the cellular levels of AMP and GMP, which are essential metabolites for DNA synthesis, RNA synthesis, energy storage, cell signaling, and other functions. 6-MP may also inhibit DNA and RNA synthesis by less well characterized mechanisms.

The major clinical application of 6-MP is in acute lymphoblastic leukemia (ALL), especially in the maintenance phase of a prolonged combination chemotherapy regimen. 6-MP is also active against normal lymphocytes and can be used as an immunosuppressive agent. For unknown reasons, the prodrug AZA is a superior immunosuppressant compared to 6-MP and is typically the drug of choice for this application. AZA is discussed in detail in Chapter 43.

Both the effectiveness and the toxicity of 6-MP are potentiated by **allopurinol.** Allopurinol inhibits xanthine oxidase, thereby preventing the oxidation of 6-MP to its inactive metabolite 6-thiouric acid. (In fact, allopurinol was discovered in an effort to inhibit the metabolism of 6-MP by xanthine oxidase.) Coadministration of allopurinol with 6-MP allows the dose of 6-MP to be reduced by 2/3 (although toxicity is proportionally increased as well). Allopurinol is often used as a single agent to prevent the hyperuricemia that could result from the destruction of cancer cells by chemotherapeutic agents (**tumor lysis syndrome**). The use of allopurinol in the treatment of gout is presented in Chapter 46.

**Pentostatin** (Fig. 31-9) is a selective inhibitor of adenosine deaminase (ADA; see above). The drug is a structural analogue of the intermediate in the reaction catalyzed by ADA, and binds to the enzyme with very high affinity. The resulting inhibition of ADA causes an increase in intracellular adenosine and 2′-deoxyadenosine levels. The increased adenosine and 2′-deoxyadenosine have multiple effects on purine nucleotide metabolism. In particular, 2′-deoxyadenosine irreversibly inhibits S-adenosylhomocysteine hydrolase, and the resulting increase in intracellular S-adenosylhomocysteine is toxic to lymphocytes. This action may account for the effectiveness of pentostatin against some leukemias and some lymphomas. Pentostatin is especially effective against hairy cell leukemia.

## INHIBITORS OF RIBONUCLEOTIDE REDUCTASE

**Hydroxyurea** inhibits ribonucleotide reductase by scavenging a tyrosyl radical at the active center of the enzyme. In the

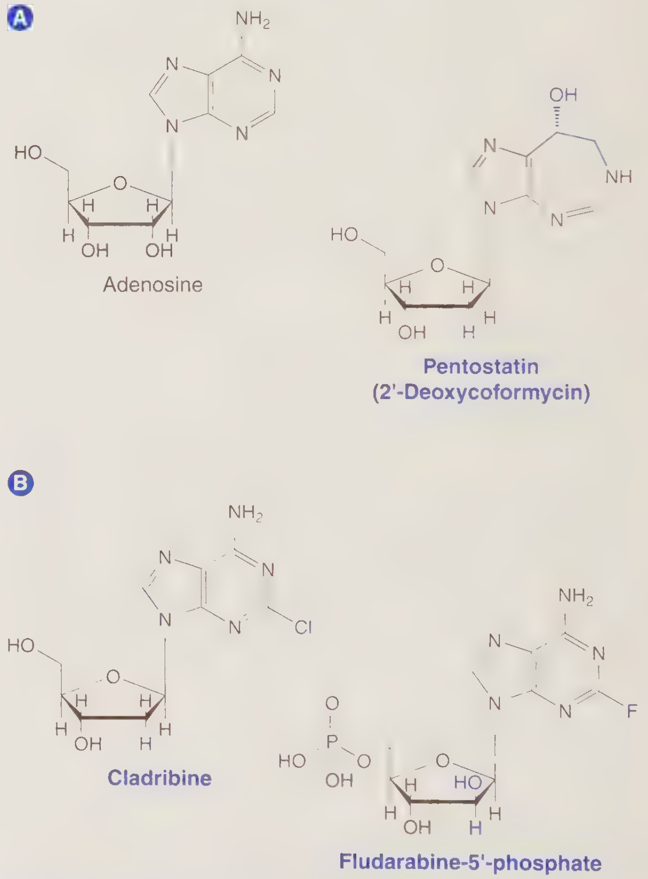


**Figure 31-9. Structures of Adenosine, Pentostatin, Cladribine, and Fludarabine.** **A.** Pentostatin inhibits adenosine deaminase (ADA), the enzyme that converts adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. Pentostatin binds to ADA with very high affinity ($K_d = 2.5 \times 10^{-12}$ M) because it structurally resembles the intermediate (or transition state, highlighted in blue) in this enzymatic reaction. **B.** Cladribine and fludarabine-5′-phosphate are also adenosine analogues. Cladribine is a chlorinated purine analogue that is incorporated into DNA and causes DNA strand breaks. Fludarabine phosphate is a fluorinated purine analogue that is incorporated into DNA and RNA; this drug also inhibits DNA polymerase and ribonucleotide reductase.

absence of this free radical, ribonucleotide reductase is unable to convert nucleotides to deoxynucleotides, and DNA synthesis is thereby inhibited.

Hydroxyurea is approved for use in the treatment of adult sickle cell disease and certain neoplastic diseases. The mechanism of action of hydroxyurea in the treatment of sickle cell disease may or may not be related to inhibition of ribonucleotide reductase. As an alternative to this mechanism, hydroxyurea has been shown to increase the expression of the fetal isoform of hemoglobin (HbF), which inhibits the polymerization of sickle hemoglobin (HbS) and, thereby, decreases red blood cell sickling under conditions of hypoxia. Hydroxyurea significantly decreases the incidence of painful (vaso-occlusive) crisis in patients with sickle cell disease. The mechanism by which hydroxyurea increases HbF production is unknown. The role of hydroxyurea in the treatment of sickle cell anemia is discussed further in Chapter 42, Pharmacology of Hematopoietic and Immune Cell Stimulation.

The neoplastic applications of hydroxyurea include head and neck cancers and myeloproliferative disorders such as polycythemia vera and essential thrombocytosis. In head and neck cancers, hydroxyurea is used as a radiosensitizing agent (i.e., an agent that increases the effectiveness of radiation therapy). The mechanism of radiosensitization is unknown; current theories suggest that hydroxyurea could increase tumor cell sensitivity to radiation by decreasing DNA repair or by synchronizing the cell cycle timing of tumor cells. In myeloproliferative disorders, hydroxyurea can be used as a single agent or in combination with other agents to inhibit the excessive growth of myeloid cells in the bone marrow. The applications of hydroxyurea for these indications have been limited somewhat by concerns that long-term hydroxyurea use may be leukemogenic.

## PURINE AND PYRIMIDINE ANALOGUES THAT ARE INCORPORATED INTO DNA

A number of antimetabolites exert their major therapeutic effect by acting as "rogue" nucleotides. These drugs are substrates for the various pathways of nucleotide metabolism, including ribosylation, ribonucleotide reduction, and nucleoside and nucleotide phosphorylation. The sugar triphosphate forms of these drugs can then be incorporated into DNA. Once incorporated into DNA, the structural differences between these compounds and their natural counterparts disrupt the structure of DNA, resulting in DNA chain termination, DNA strand breakage, and inhibition of cell growth. A number of other drugs (e.g., alkylating agents) that induce DNA strand breakage are discussed in Chapter 32; unlike the drugs discussed in Chapter 32, the agents discussed here rely on nucleotide metabolism pathways for their effects.

**Thioguanine** is a guanine analogue in which a sulfur atom replaces the keto group at C-6 of the purine ring (Fig. 31-8). As with mercaptopurine, thioguanine is converted by HGPRT to its nucleotide form, 6-thioguanosine-5′-monophosphate (6-thioGMP). Unlike T-IMP, the nucleotide form of mercaptopurine, 6-thioGMP is a good substrate for guanylyl kinase, the enzyme that catalyzes the conversion of GMP to GTP. By this mechanism, 6-thioGMP is converted to 6-thioGTP, which is incorporated into DNA. Within the structure of DNA, 6-thioGTP interferes with RNA transcription and DNA replication, resulting in cell death. 6-ThioGMP also irreversibly inhibits inosine monophosphate dehydrogenase (IMPDH) and, thereby, depletes cellular pools of GMP (Fig. 31-3).

Thioguanine is used in the treatment of acute myelocytic leukemia. Major side effects of thioguanine include bone marrow suppression and gastrointestinal toxicity.

**Fludarabine phosphate** (Fig. 31-9) is a fluorinated purine nucleotide analogue that is structurally related to the antiviral agent vidarabine (see Chapter 34, Pharmacology of Viral Replication). The triphosphate form of fludarabine is incorporated into DNA and RNA, causing DNA chain termination and inhibition of RNA function. Fludarabine triphosphate also inhibits DNA polymerase and ribonucleotide reductase and, thereby, decreases nucleotide and nucleic acid synthesis in cells. The relative importance of these actions in mediating the cellular toxicity of the drug remains to be elucidated. Fludarabine phosphate is used in the treatment of lymphoproliferative disorders, especially chronic lymphocytic leukemia (CLL) and low-grade B-cell lymphomas.

**Cladribine** is a chlorinated purine analogue that is structurally related to fludarabine phosphate (Fig. 31-9). Cladribine triphosphate is incorporated into DNA, causing strand breaks. Cladribine also depletes intracellular pools of the essential purine metabolites NAD and ATP. Cladribine is approved for use in the treatment of hairy cell leukemia, and has been used experimentally in the treatment of other types of leukemia and lymphoma.

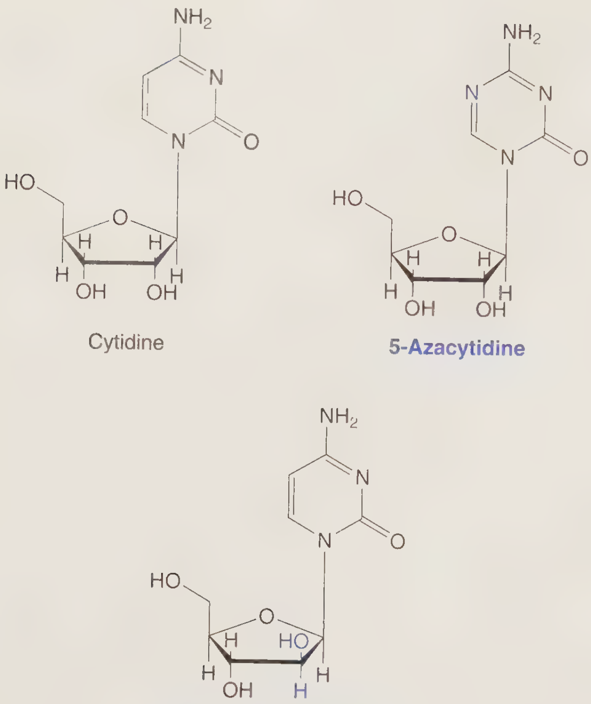
**Cytarabine (araC)** is a cytidine analogue that is metabolized to araCTP (Fig. 31-10). AraCTP competes with CTP for DNA polymerase, and incorporation of araCTP into DNA results in chain termination and cell death (Fig. 31-4). Synergism between cytarabine and cyclophosphamide has been noted, presumably because of the reduced DNA repair caused by cytarabine's inhibition of DNA polymerase. Cytarabine is used to induce and maintain remission in acute myelocytic leukemia; it is especially effective when combined with an anthracycline for this indication.

**5-Azacytidine** is a cytidine analogue whose triphosphate metabolite is incorporated into DNA and RNA (Fig. 31-10). Once incorporated into DNA, azacytidine interferes with cytosine methylation and causes cell death. Azacytidine is currently under investigation for use in the treatment of acute lymphocytic leukemia, acute myelocytic leukemia, and myelodysplastic syndromes.

## *Conclusion and Future Directions*

Antimetabolites are analogues of endogenous folates, purines, and pyrimidines that inhibit a number of enzymes involved in nucleotide synthesis. These agents have a broad spectrum of clinical applications, especially in antimicrobial and antineoplastic pharmacology. Their structural similarity to endogenous metabolites is responsible for their efficacy, as well as for the severe side effects that can accompany their use.

The antibacterial inhibitors of folate synthesis, sulfamethoxazole and trimethoprim, remain useful in the treatment of certain infections, although the development of drug resistance has decreased their clinical utility. Drug resistance mechanisms also hamper the clinical use of the antiparasitic agents pyrimethamine and sulfadiazine. Flucytosine has proved



Cytidine

**5-Azacytidine**

**Cytosine arabinoside (cytarabine, AraC)**

**Figure 31-10. Structures of Cytidine, Cytarabine, and Azacytidine.** Cytarabine and azacytidine are both analogues of the nucleoside cytidine. Cytarabine has an arabinose sugar in place of ribose (note the chirality of the hydroxyl group highlighted in blue). The incorporation of cytarabine triphosphate (araCTP) into DNA inhibits further nucleic acid synthesis, because the presence of arabinose in place of 2′-deoxyribose interrupts strand elongation. Azacytidine has an azide group (highlighted in blue) within the pyrimidine ring; this drug is incorporated into nucleic acids and interferes with the methylation of cytosine bases.

to be highly effective against fungal infections. Fluorouracil and methotrexate are useful agents against solid tumors, whereas a number of purine and pyrimidine analogues are effective against leukemias and lymphomas.

Although the major biochemical pathways involved in nucleotide synthesis have been defined, much remains to be learned about the structural diversity of these enzymes. Future prospects for the pharmacology of nucleotide metabolism include a more comprehensive definition of the selectivity and tissue specificity of the various metabolic pathways. For example, selective targeting of a particular isoform of an enzyme involved in nucleotide synthesis could allow greater target specificity and, thereby, limit drug side effects. Rational drug design is likely to guide this progress, as the structural basis for variations in enzyme isoforms could allow the development of inhibitors that preferentially target particular isoforms of the enzyme.

## *Suggested Readings*

Berg JM, Tymoczko JL, Stryer L. Biosynthesis of nucleotides. In: Biochemistry. 5th ed. New York, NY: WH Freeman & Company, 2002. (*Detailed discussion of nucleotide synthesis.*)

Allison AC. Immunosuppressive drugs: the first 50 years and a glance forward. Immunopharmacology 2000;47:63–83. (*A review of antimetabolites is included in this review of immunosuppressive drugs.*)

Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, et al. Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol Ther 2000;87:227–253. (*Discussion of synergistic interactions involving antimetabolites.*)

Hatse S, De Clercq E, Balzarini J. Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. Biochem Pharmaco1999;58:539–555. (*Discussion of the effects of purine and pyrimidine antimetabolites on tumor cell growth.*)

## Drug Summary Table Pharmacology of Nucleotide Synthesis

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF DIHYDROPTEROATE SYNTHASE**<br>*Mechanism—↓ folate synthesis by inhibiting dihydropteroate synthase* | | | | |
| *Sulfonamides:* | | | | |
| **Sulfamethoxazole** | Bacterial infections | Kernicterus in newborns | Used with trimethoprim for synergy | Bacteriostatic |
| **Sulfadiazine** | Parasitic infections | | Used with pyrimethamine for synergy | |
| *Sulfones:* | | | | |
| **Dapsone** | *P. carinii* pneumonia<br>Leprosy | | Synergistic with trimethoprim or pyrimethamine | |
| **INHIBITORS OF DIHYDROFOLATE REDUCTASE (DHFR)**<br>*Mechanism—↓ folate synthesis by inhibiting DHFR* | | | | |
| **Trimethoprim** | Urinary tract infections<br>Other infections (in combination with sulfas) | | Synergistic with sulfas | Bacteriostatic<br>Excreted unchanged in urine (allows use in UTIs) |
| **Pyrimethamine** | Parasitic infections<br>Malaria | | | Substantial malarial resistance exists (see Chapter 37) |
| **Methotrexate** | Cancer chemotherapy<br>Immunosuppression<br>Psoriasis<br>Ectopic pregnancy termination | Myelosuppression<br>Gastrointestinal (GI) toxicity | Toxic to the fetus—causes neural tube defects | Can be used in high doses with folinic acid rescue for cancer chemotherapy |
| **INHIBITORS OF THYMIDYLATE SYNTHASE**<br>*Mechanism—Prevent dTMP synthesis by inhibiting thymidylate synthase. Also interfere with protein synthesis after FdUMP (drug metabolite) is incorporated into mRNA.* | | | | |
| **Fluorouracil (5-FU)** | Breast and GI cancers | Myelosuppression<br>GI toxicity | | Folinic acid can be used to potentiate the action of 5-FU |
| **Flucytosine** | Fungal infections | Myelosuppression<br>GI toxicity | | Converted to 5-FU by fungal cytosine deaminase |

(Continued)

## Drug Summary Table Pharmacology of Nucleotide Synthesis *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF PURINE METABOLISM** | | | | |
| *Mechanism—Drug metabolites inhibit IMPDH and other synthetic enzymes, thereby interfering with AMP and GMP synthesis* | | | | |
| **Mercaptopurine (6-MP)** | Leukemia (especially acute lymphoblastic leukemia) | Myelosuppression, GI toxicity | Effectiveness and toxicity increased by allopurinol | |
| **Azathioprine (AZA)** | Immunosuppression | Myelosuppression, GI toxicity | | Less toxic prodrug form of 6-MP |
| **Pentostatin** | Hairy cell leukemia<br>T cell lymphomas | Myelosuppression, GI toxicity | | Selective inhibitor of adenosine deaminase (ADA) |
| **INHIBITORS OF RIBONUCLEOTIDE REDUCTASE** | | | | |
| *Mechanism—Inhibit ribonucleotide reductase, the enzyme that converts ribonucleotides to deoxyribonucleotides* | | | | |
| **Hydroxyurea** | Hematologic malignancies<br>Sickle cell anemia (adults only) | Myelosuppression, GI toxicity | Cytarabine, fluorouracil | Reduces tyrosine free radical critical to mechanism of action of ribonucleotide reductase |
| **PURINE AND PYRIMIDINE ANALOGUES THAT ARE INCORPORATED INTO DNA** | | | | |
| *Mechanism—Incorporation into DNA and RNA results in inhibition of DNA polymerase, thereby causing cell death* | | | | |
| **Thioguanine** | Acute myelocytic leukemia | Myelosuppression, GI toxicity | | Guanine analogue |
| **Fludarabine phosphate** | Chronic lymphocytic leukemia, B cell lymphomas | Myelosuppression, GI toxicity | | Purine nucleotide analogue |
| **Cladribine** | Hairy cell leukemia | Myelosuppression, GI toxicity | | Purine analogue |
| **Cytarabine (araC)** | Acute myelocytic leukemia | Myelosuppression, GI toxicity | | Cytidine analogue |
| **Azacytidine** | Investigational | Myelosuppression, GI toxicity | | Cytidine analogue |

# 32

# Pharmacology of DNA Replication and Mitosis

Andrea S. Wolf and David A. Frank

**Introduction**
**Case**
**Biochemistry of DNA Replication and Mitosis**
  DNA Structure
  DNA Replication and Supercoiling
  Microtubules and Mitosis
**Pharmacologic Classes and Agents**
  Agents That Directly Modify DNA Structure
    *Alkylating Agents*
    *Platinum Compounds*
    *Bleomycin*
  Topoisomerase Inhibitors
    *Camptothecins*
    *Anthracyclines*
    *Epipodophyllotoxins*
    *Amsacrine*
    *Antibacterial Topoisomerase Inhibitors: Quinolones*
  Microtubule Inhibitors
    *Inhibitors of Microtubule Polymerization*
      *Vinca Alkaloids*
      *Colchicine*
      *Griseofulvin*
    *Inhibitors of Microtubule Depolymerization: Taxanes*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

The previous chapter discussed the biochemistry of nucleotide synthesis and metabolism, and the pharmacologic strategies used to interrupt those processes. This chapter discusses the biochemistry and pharmacology of three processes that are also critical for cell growth and division: the assembly of nucleotides to form DNA, the division of a single cell into two identical daughter cells (mitosis), and the maintenance of DNA structure during DNA replication.

The drugs discussed in this chapter are useful for treating cancer and microbial infections, as well as for suppressing harmful immune responses. These drugs cause cellular damage by intercalating into the DNA polymer and disrupting its structure; by interfering with the action of enzymes that are critical to the proper polymerization, winding, and transcription of nucleic acids; and by inhibiting the activity of microtubules, which are necessary for the proper separation of chromosomes during mitosis. As a result, all of these agents interrupt cell growth and division, and the antineoplastic agents initiate cellular signaling cascades leading to programmed cell death (apoptosis).

## ■ Case

One day, J.L., a 23-year-old graduate student who had previously been in good health, noticed a hard lump in his left testis while showering. Concerned by the finding, J.L.'s physician ordered an ultrasound examination, which showed a solid mass suggestive of cancer. The testis was removed surgically; pathologic review confirmed the diagnosis of testicular cancer. A chest x-ray revealed several lung nodules, which were thought to represent metastatic spread of the cancer. J.L. was treated with several cycles of combination chemotherapy, including bleomycin, etoposide, and cisplatin. The lung nodules disappeared completely. One year later, J.L. was able to resume his studies, and there were no signs of recurrence of the cancer. Nonetheless, at every subsequent follow-up visit, J.L.'s physician asks him whether he is developing shortness of breath.

### QUESTIONS

■ **1.** What is the molecular target of each of the drugs in J.L.'s combination chemotherapy regimen?

2. By what mechanisms could etoposide, bleomycin, and cisplatin act synergistically against J.L.'s testicular cancer?
3. Why does J.L.'s physician inquire about shortness of breath at each follow-up visit?
4. How did serendipity lead to the discovery of cisplatin, the most efficacious drug against testicular cancer?

## BIOCHEMISTRY OF DNA REPLICATION AND MITOSIS

### DNA STRUCTURE

As reported by Watson and Crick in 1953, DNA is composed of two strands of polymerized deoxyribonucleotides that wind around one another in a "double helix" conformation. The 5′-hydroxyl group of each nucleotide's deoxyribose ring is joined by a phosphate group to the 3′-hydroxyl group of the next nucleotide, thereby forming the phosphodiester backbone of each side of the double helical "ladder" (Figs. 32-1 and 32-2). The purines **adenine** (A) and **guanine** (G) and the pyrimidines **thymine** (T) and **cytosine** (C), which are covalently linked to the deoxyribose ring, associate with one another (A with T, G with C) via hydrogen bonds to form the "rungs" of the ladder (Fig. 32-2). It is the linear sequence of bases that encodes the genetic information of a cell. Several atoms in the DNA molecule, particularly $N^7$ and $O^6$ of guanine, $N^3$ of cytosine, and $N^1$ and $N^3$ of adenine, possess a partial negative charge and can, therefore, act as **nucleophiles** (Fig. 32-3). Many DNA damaging agents are **electrophilic;**

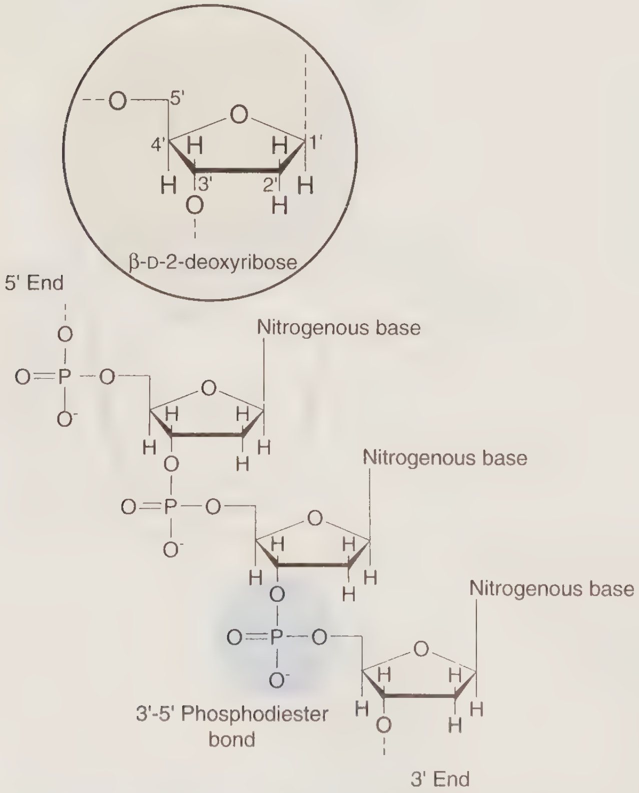


Figure 32-1. **Backbone Structure of DNA.** DNA is a nucleotide polymer oriented so that a phosphodiester bond connects the 2′-deoxyribose sugars of each neighboring nucleotide. The phosphodiester bond links the 3′-OH of one deoxyribose to the 5′-OH of the next deoxyribose, thus forming the backbone of the DNA strand.

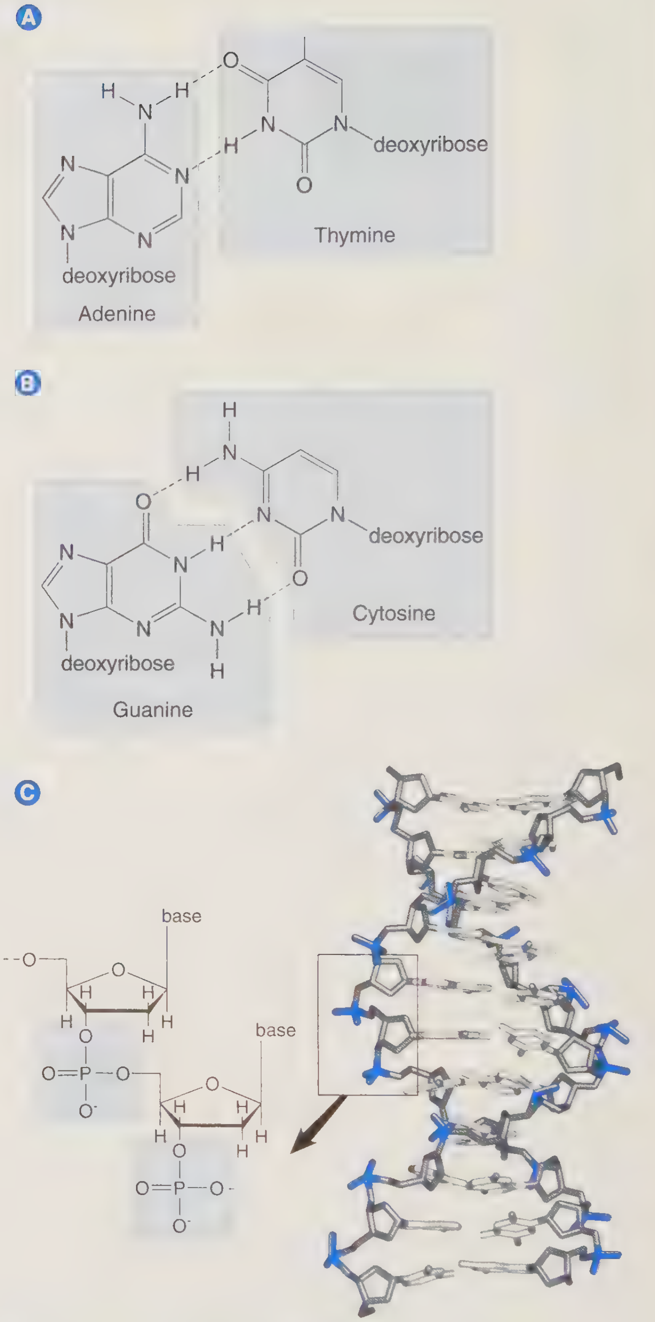


Figure 32-2. **Hydrogen Bonding Between DNA Strands.** **A** and **B.** The indicated hydrogen bonds (dashed lines) between complementary bases on opposite DNA strands show that adenine (A) and thymine (T) form two hydrogen bonds, while guanine (G) and cytosine (C) form three hydrogen bonds. **C.** These nitrogenous A-T and G-C base pairs form the "rungs" of the DNA double helical "ladder." Note that the deoxyribose moieties and the phosphodiester bonds are located on the outside of the DNA molecule, while the purine and pyrimidine bases stack in the center of the DNA ladder.

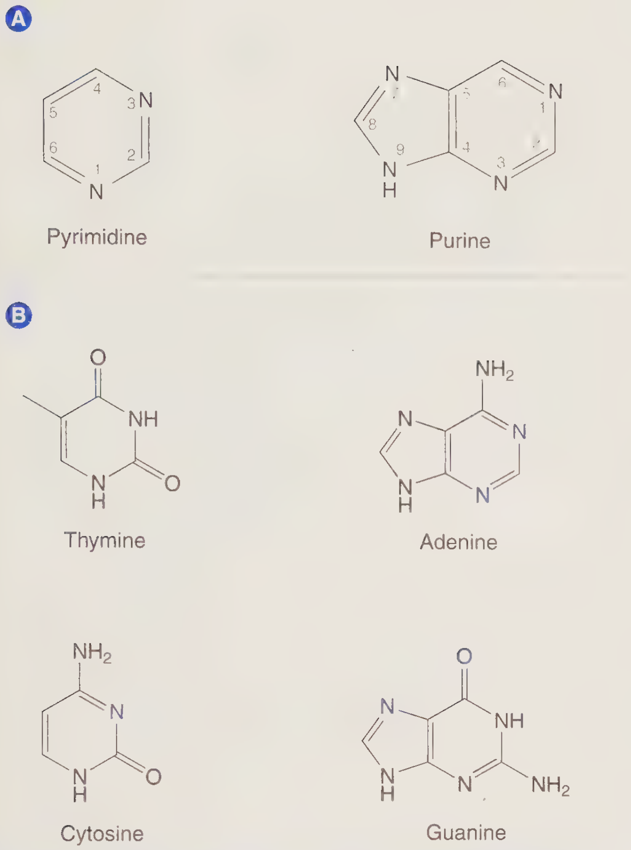


**Figure 32-3. Chemical Structures of Purines and Pyrimidines.** **A.** The purine and pyrimidine skeletons form the structural basis for all bases, nucleosides and nucleotides. **B.** Thymine and cytosine are pyrimidines, while adenine and guanine are purines. The chemical structures of the nitrogenous bases impart areas of partial negative charge (indicated by blue highlighting). These areas of partial negative charge are potentially nucleophilic, and are, therefore, sites that can be attacked by such electrophilic compounds as alkylating agents.

these agents chemically modify nucleic acids at their nucleophilic centers, creating a covalent bond between DNA and the DNA-damaging drug. It is this covalent binding that interferes with the structure and function of DNA and, ultimately, leads to cell death.

## DNA REPLICATION AND SUPERCOILING

DNA replication is critical for cell division in eukaryotes and prokaryotes. Although DNA replication involves several steps, it is important to appreciate the role of the enzyme **topoisomerase** to understand the mechanisms by which several of the classes of agents discussed here interrupt the cell cycle.

During DNA replication, complementary strands of DNA are synthesized bidirectionally, forming two so-called replication forks. To initiate this process, the two DNA strands that comprise the double helix must unwind and separate. In so doing, the DNA strands form "**supercoils**" in which the helical polymer overtwists as it rotates in the same direction as the turn of the helix. (This is similar to what happens to telephone cords during use.) Supercoils increase tension in DNA strands and, thereby, interfere with further unwinding. In the absence of a process to relieve the stress created by the supercoils, the entire chromosome would have to rotate; this process would be complex and energy-consuming, and could entangle the entire molecule.

Fortunately, one class of DNA replication enzymes—the topoisomerases—is capable of removing excess DNA supercoils. **DNA topoisomerases** modulate the degree of supercoiling by breaking, rotating, and religating DNA strands. These enzymes are capable of establishing degrees of supercoiling that are positive (coils with an increased number of turns relative to relaxed DNA) or negative (coils with fewer turns than relaxed DNA) as required to accommodate the processes of replication and transcription. **Type I topoisomerase** forms and reseals single-stranded breaks in DNA to decrease positive supercoiling (Fig. 32-4). **Type II topoisomerase** performs these nuclease and ligase operations on both strands of DNA (Fig. 32-5). Type II topoisomerase is both more complex and more versatile than type I topoisomerase, and the type II enzyme serves as a more frequent molecular target for chemotherapeutic agents. The mechanism of action of type II topoisomerase proceeds in two steps. First, the enzyme binds a segment of DNA and forms covalent bonds with phosphates from each strand, thereby nicking both strands. Second, the enzyme causes a second stretch of DNA from the same molecule to pass through the break, either relieving or generating positive supercoils depending on the direction from which the second stretch of DNA is brought (Fig. 32-5).

**DNA gyrase** is a bacterial type II topoisomerase that can introduce negative supercoils before the DNA strands separate and, thereby, neutralize positive supercoils that form as the strands unwind. In most prokaryotes, DNA molecules are circular and form interlocked segments after chromosomal replication. Because DNA gyrase is required to unknot these segments, the type II topoisomerases are particularly important in prokaryotic reproduction.

## MICROTUBULES AND MITOSIS

Once a cell has replicated its DNA, the cell is competent to undergo mitosis. In this process, a single cell divides into two identical daughter cells. The cell cycle transitions from DNA replication (S phase) to G2 phase and then to mitosis (M phase) are complex, and depend on the coordinated action of a number of so-called cyclin-dependent kinases (CDKs, see Chapter 30, Principles of Antimicrobial and Antineoplastic Pharmacology). Many cancer cells exhibit dysregulation of cell cycle timing. The biochemical control of the cell cycle transitions between DNA replication and the initiation of mitosis is an active area of cancer research; it is hoped that, in the near future, cyclins will become pharmacologic targets for antineoplastic chemotherapy. Currently, however, microtubules are the only pharmacologically relevant structures that are targeted in mitosis.

Microtubules are cylindrical, hollow fibers composed of polymers of tubulin, which is a heterodimeric protein consisting of α-tubulin and β-tubulin subunits (Fig. 32-6). α-Tubulin and β-tubulin are encoded by separate genes, but they have similar three-dimensional structures. Both α- and β-tubulin bind GTP; in addition, β-tubulin (but not α-tubulin) can hydrolyze GTP to GDP, as described below. Microtubules

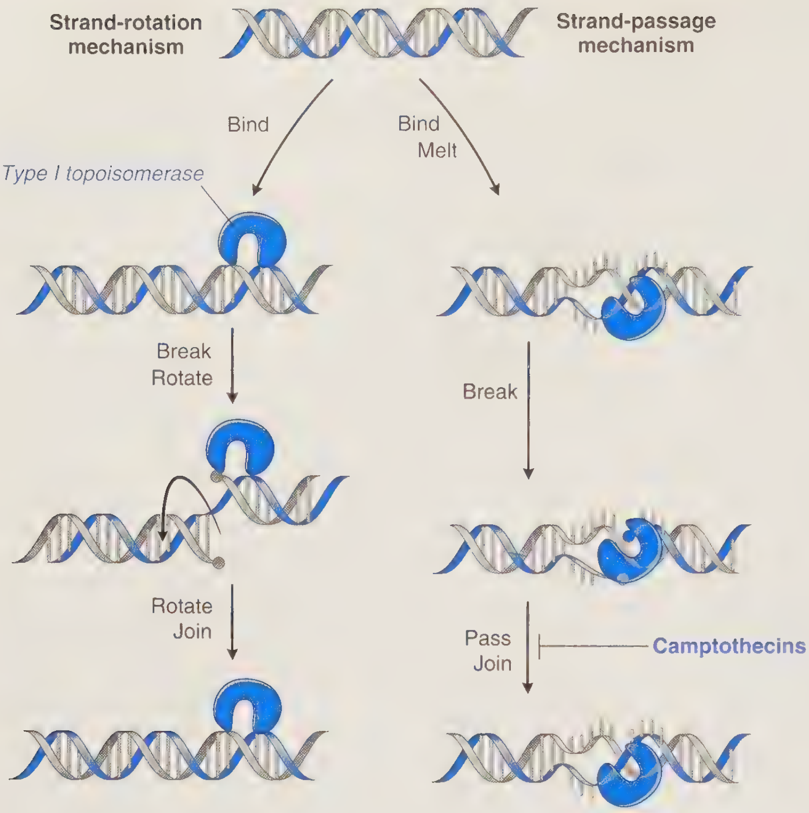

Strand-rotation mechanism
Strand-passage mechanism
Bind
Bind
Melt
Type I topoisomerase
Break
Rotate
Break
Rotate
Join
Pass
Join
Camptothecins

**Figure 32-4. Regulation of DNA Supercoiling by Type I Topoisomerases.** Two mechanisms have been proposed for the action of type I topoisomerases. In the strand-rotation model, type I topoisomerase binds to opposite strands of the DNA double helix. The topoisomerase then nicks one strand, and remains bound to one of the nicked ends (denoted by a filled gray circle). The unbound end of the nicked strand is able to unwind by one or more turns, and is then joined (religated) to its parent strand. In the strand-passage model, type I topoisomerase binding to the DNA double helix results in melting (separation) of the two DNA strands. The DNA-bound topoisomerase then introduces a nick into one strand, while remaining bound to each end of the broken DNA strand (denoted by filled blue circles). The broken strand is then passed through the helix and joined (religated), resulting in a net unwinding of the DNA. Camptothecins inhibit the joining of the broken strand of DNA after strand passage.

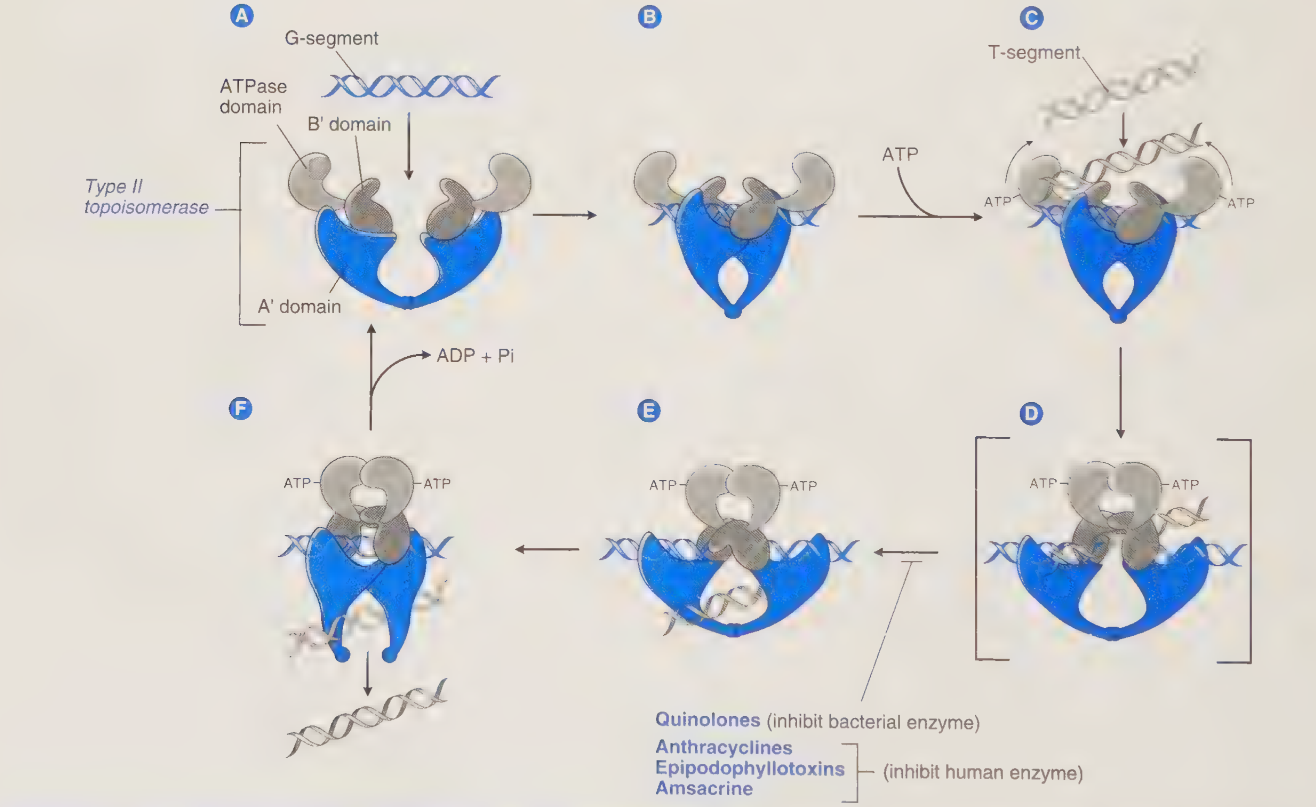

A
G-segment
ATPase domain
B' domain
Type II topoisomerase
A' domain
B
ATP
C
T-segment
ATP
ATP
ADP + Pi
F
ATP
ATP
E
ATP
ATP
D
ATP
ATP
Quinolones (inhibit bacterial enzyme)
Anthracyclines
Epipodophyllotoxins
Amsacrine
(inhibit human enzyme)

**Figure 32-5. Regulation of DNA Supercoiling by Type II Topoisomerases.** **A.** The type II topoisomerase enzyme contains A′, B′, and ATPase domains. The A′ and B′ domains engage a segment of the DNA double helix (G-segment). **B.** Interaction with the G-segment induces a conformational change in the type II isomerase, causing it to "lock" around the DNA G-segment. **C.** ATP binds to the ATPase domains of the topoisomerase, and a second segment of the DNA double helix (T-segment) enters and is "locked" into the B′ domains. **D.** Once the enzyme is engaged with both DNA segments, the topoismerase cuts both strands of the G-segment DNA. **E.** This double-stranded nick in the G-segment allows the T-segment to pass through the G-segment to the opposite side of the topoisomerase. **F.** The T-segment is released from the topoisomerase, and the G-segment nick is religated. ATP is hydrolyzed to ADP, ADP dissociates from the topoisomerase, and the cycle can begin anew. The result of each cycle is to change the coiling number of DNA by one. Quinolones, anthracyclines, epipodophyllotoxins, and amsacrine inhibit the passage of the T-segment and the religation of the nicked G-segment.

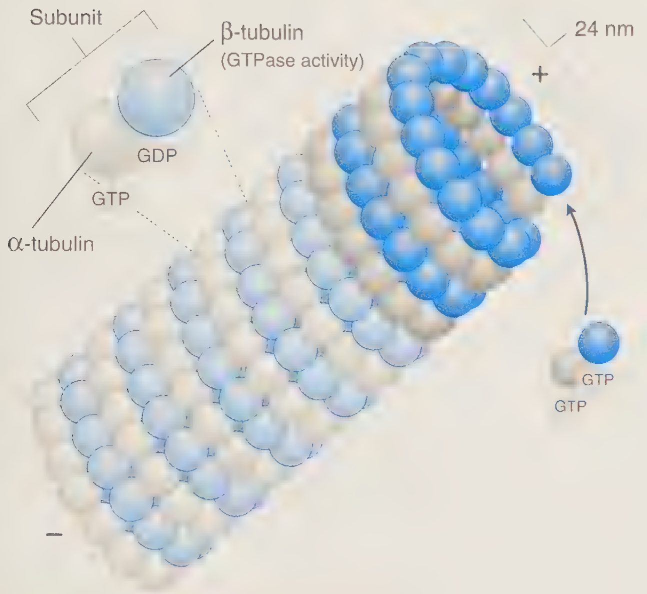


**Figure 32-6. Microtubule Structure.** Microtubules are hollow cylindrical tubes that polymerize from tubulin subunits. Each tubulin subunit is a heterodimer composed of α-tubulin (shades of gray) and β-tubulin (shades of blue). Both α-tubulin and β-tubulin bind GTP (dark shades of gray and blue); β-tubulin hydrolyzes GTP to GDP after the tubulin subunit is added to the end of a microtubule (lighter shades of gray and blue). Microtubules are dynamic structures that grow and shrink lengthwise; the cylindrical tubes are composed of 13 subunits arranged concentrically, resulting in a diameter of 24 nm. Note that microtubules have an inherent structural asymmetry. One end of a microtubule is limited by α-tubulin, and is referred to as the negative (–) end; the opposite end is limited by β-tubulin, and is referred to as the positive (+) end.

originate from a central microtubule organizing center (the centrosome, which includes two centrioles and associated proteins), where γ-tubulin (a protein with homology to α-tubulin and β-tubulin) nucleates tubulin polymerization. Nascent microtubules assemble into protofilaments, which are longitudinal polymers of tubulin. Each protofilament interacts laterally with two other protofilaments to form a hollow-core tube, 24 nm in diameter, that consists of 13 protofilaments arranged concentrically. *Because tubulin is a heterodimer, this tube has inherent asymmetry;* the end of a microtubule nearest the centrosome is bordered by α-tubulin and is called the (–) end, while the end of a microtubule extending from the centrosome is bordered by β-tubulin and is called the (+) end (Fig. 32-6). Tubulin is added at different rates to the (–) and (+) ends; the (+) end grows (adds tubulin) twice as fast as the (–) end.

Microtubules are not static structures. Rather, they possess an inherent property known as **dynamic instability** (Fig. 32-7). Tubulin heterodimers add to the end of the microtubule with GTP bound to both the α-tubulin and the β-tubulin subunits. As the microtubule grows, the β-tubulin of each tubulin heterodimer hydrolyzes its GTP to GDP. The hydrolysis of GTP to GDP introduces a conformational change in the tubulin that destabilizes the microtubule. The exact mechanism of this destabilization is unknown, but it may be related to a decrease in the strength of lateral protofilament interactions or an increase in the tendency for protofilaments to "curve" away from the straight microtubule.

Therefore, the rate of microtubule polymerization relative to the rate of GTP hydrolysis by β-tubulin determines microtubule stability. If a microtubule polymerizes tubulin faster than β-tubulin hydrolyzes GTP to GDP, then, in the steady state, there is a cap of GTP-bound β-tubulin at the (+) end of the microtubule. This GTP cap provides stability to the microtubule structure, allowing further polymerization of the microtubule. Conversely, if tubulin polymerization proceeds more slowly than the hydrolysis of GTP to GDP by β-tubulin, then, in the steady state, the (+) end of the microtubule is enriched with GDP-bound β-tubulin. This conformation of the tubulin subunits is unstable and causes rapid depolymerization of the microtubule. The ability of microtubules to assemble and disassemble rapidly is important for their many physiologic roles. As discussed below, pharmacologic agents can disrupt microtubule function either by preventing the assembly of tubulin into microtubules or by stabilizing existing microtubules (and, thereby, preventing microtubule disassembly).

Microtubules have important physiologic roles in mitosis, intracellular protein trafficking, vesicular movement, and cell structure and shape. Mitosis is the physiologic role that is targeted pharmacologically; the other physiologic roles, however, predict many of the toxic effects of drugs that interrupt microtubule function.

Recall that microtubules nucleate from centrosomes, which consist of centrioles and other associated proteins. In mitosis, the two centrosomes align at opposite ends of the cell. Microtubules are extremely dynamic during M phase; they grow and shrink during M phase at rates much greater than those during other phases of the cell cycle. This increased dynamic instability during M phase allows microtubules to locate and attach to the chromosomes. The microtubules emanating from each centrosome bind to kinetochores, which are proteins that attach to the centomere of a chromosome. Once the kinetochore of each chromosome is attached to a microtubule, microtubule-associated proteins act as motors to align the kinetochore-bound chromosomes at the equator of the cell (defined by the midpoint between the two centrosomes). When every chromosome has aligned at the equator, the microtubules shorten, separating a diploid pair of chromosomes into each half of the cell. Finally, cytokinesis (division of the cytoplasm) occurs, and two daughter cells are formed. Although numerous other proteins are involved in the regulation of mitosis, microtubules have a critical role in the process. Disruption of microtubule function freezes cells in M phase, leading eventually to the activation of programmed cell death (apoptosis).

## PHARMACOLOGIC CLASSES AND AGENTS

There are currently three general classes of drugs that interrupt DNA replication and mitosis. First, agents that directly damage DNA prevent the precise fidelity required to maintain a stable genome between generations. These agents chemically target structural aspects of DNA, and disrupt the three-dimensional structure of this macromolecule. Second, topoisomerase inhibitors prevent DNA from uncoiling and/or "lock" the topoisomerase enzymes in a state that leaves the DNA strands broken. The end result is much the same as that caused by agents that directly damage DNA: the disruption

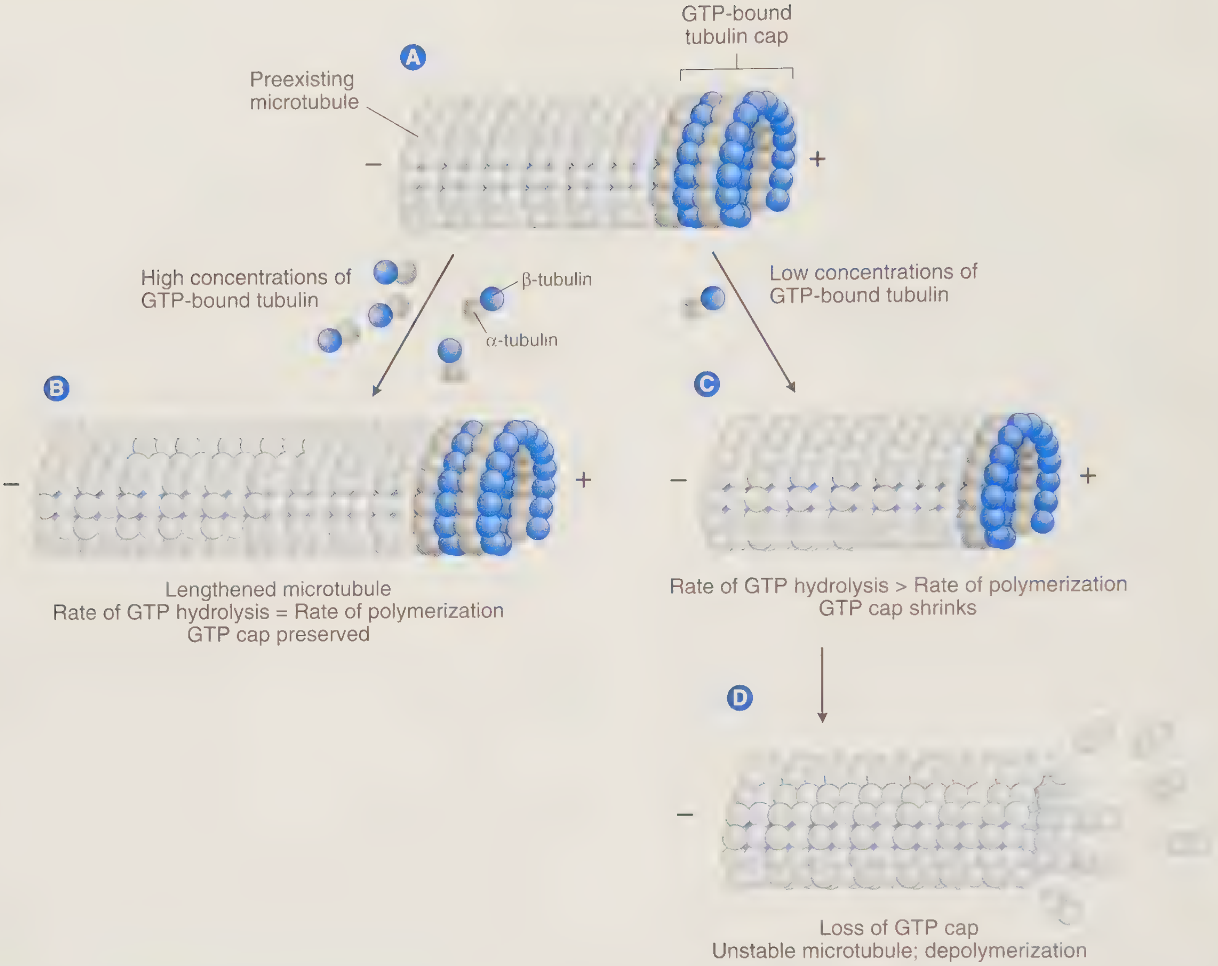

A
Preexisting microtubule
GTP-bound tubulin cap
−
+
High concentrations of GTP-bound tubulin
β-tubulin
α-tubulin
Low concentrations of GTP-bound tubulin
B
−
+
Lengthened microtubule
Rate of GTP hydrolysis = Rate of polymerization
GTP cap preserved
C
−
+
Rate of GTP hydrolysis > Rate of polymerization
GTP cap shrinks
D
−
Loss of GTP cap
Unstable microtubule; depolymerization

**Figure 32-7. Dynamic Instability of Microtubules.** **A.** A pre-existing microtubule is characterized by tubulin subunits that have predominantly hydrolyzed the GTP on β-tubulin to GDP (light gray and light blue). However, β-tubulin subunits that have recently been added to the microtubule have not yet hydrolyzed GTP (dark gray and dark blue). The GTP-bound tubulin subunits form a GTP-bound tubulin cap at the end of the microtubule. **B.** In the presence of a high concentration of GTP-bound free tubulin subunits, new GTP-bound tubulin is added to the (+) end of the microtubule at a rate that equals or exceeds the rate of GTP hydrolysis by β-tubulin. Maintenance of a GTP-bound tubulin cap results in a stable microtubule. **C.** In the presence of a low concentration of GTP-bound free tubulin subunits, new GTP-bound tubulin is added to the (+) end of the microtubule at a rate less than the rate of GTP hydrolysis by β-tubulin. This results in shrinkage of the GTP-bound tubulin cap. **D.** A microtubule that lacks a GTP-bound tubulin cap is unstable, and undergoes depolymerization.

of DNA structure initiates apoptosis and cell death. Third, microtubule inhibitors disrupt the dynamic instability required for proper microtubule function. Without functioning microtubules, a cell cannot undergo mitosis.

## AGENTS THAT DIRECTLY MODIFY DNA STRUCTURE

### Alkylating Agents

The advent of modern chemotherapy dates to the 1940s, when highly reactive alkylating agents were first noted to induce remissions in otherwise untreatable malignancies. The clinical use of these agents was sparked by observations in sailors who had inadvertently been exposed to nitrogen mustards during World War II. These men were found to have dramatic suppression of their hematopoietic cells, suggesting that alkylating agents could have therapeutic utility in such blood-derived malignancies as leukemias and lymphomas. Soon thereafter, it was suggested that alkylating agents could also be useful in treating epithelial tumors, mesenchymal tumors, carcinomas, and sarcomas; in fact, alkylating agents are commonly used against all of these diseases today.

Alkylating agents such as **cyclophosphamide, mechlorethamine, melphalan, chlorambucil,** and **thiotepa** are electrophilic molecules that covalently attach an alkyl group to nucleophilic sites on DNA. Depending on the particular agent, alkylation can occur on nitrogen or oxygen atoms of the base, the phosphate backbone, or a DNA-associated protein. Alkylating agents typically have two strong leaving groups (Fig. 32-8). This structure confers the ability to ***bis*-alkylate,** or to alkylate a molecule twice, enabling the agent to crosslink the DNA molecule either to itself—by linking two guanine residues, for example—or to proteins. *Bis*-alkylation (crosslinking) seems to be the major mechanism of cytotoxicity (Fig. 32-9A). Alkylation of guanine residues can also result in cleavage of the guanine imidazole ring, in abnormal base-pairing between the alkylated guanine and thymine, or in

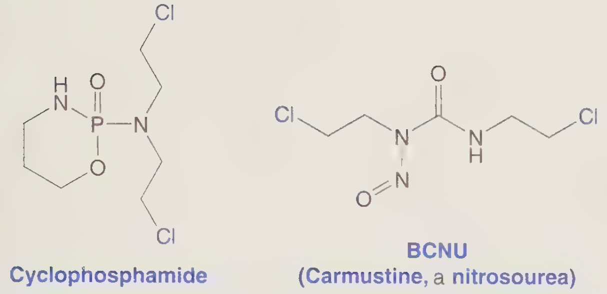


**Figure 32-8. Structures of Cyclophosphamide and BCNU.** Cyclophosphamide and BCNU (carmustine) each have two chloride leaving groups. The presence of two leaving groups allows these alkylating agents to *bis*-alkylate and, thereby, crosslink macromolecules such as DNA. The ability to crosslink DNA is crucial to the creation of DNA damage by these agents.

depurination (i.e., excision of the guanine residue) (Fig. 32-9B-D). Ring cleavage disrupts the molecular structure of DNA; anomalous DNA base-pairing causes miscoding and mutation; and depurination leads to scission of the sugar–phosphate DNA backbone. Importantly, the mutations caused by these processes can increase the risk of developing new cancers.

Although all nitrogen mustards are relatively reactive, the individual agents vary in the speed with which they react with nucleophiles; this fact has significant impact on their clinical use. Highly unstable compounds, such as mechlorethamine, cannot be administered orally because such agents alkylate target molecules within seconds to minutes. Because of this high reactivity, these molecules are powerful vesicants (causing blisters) and can severely damage skin and soft tissue if they leak outside the circulation. The rapid reactivity of alky-

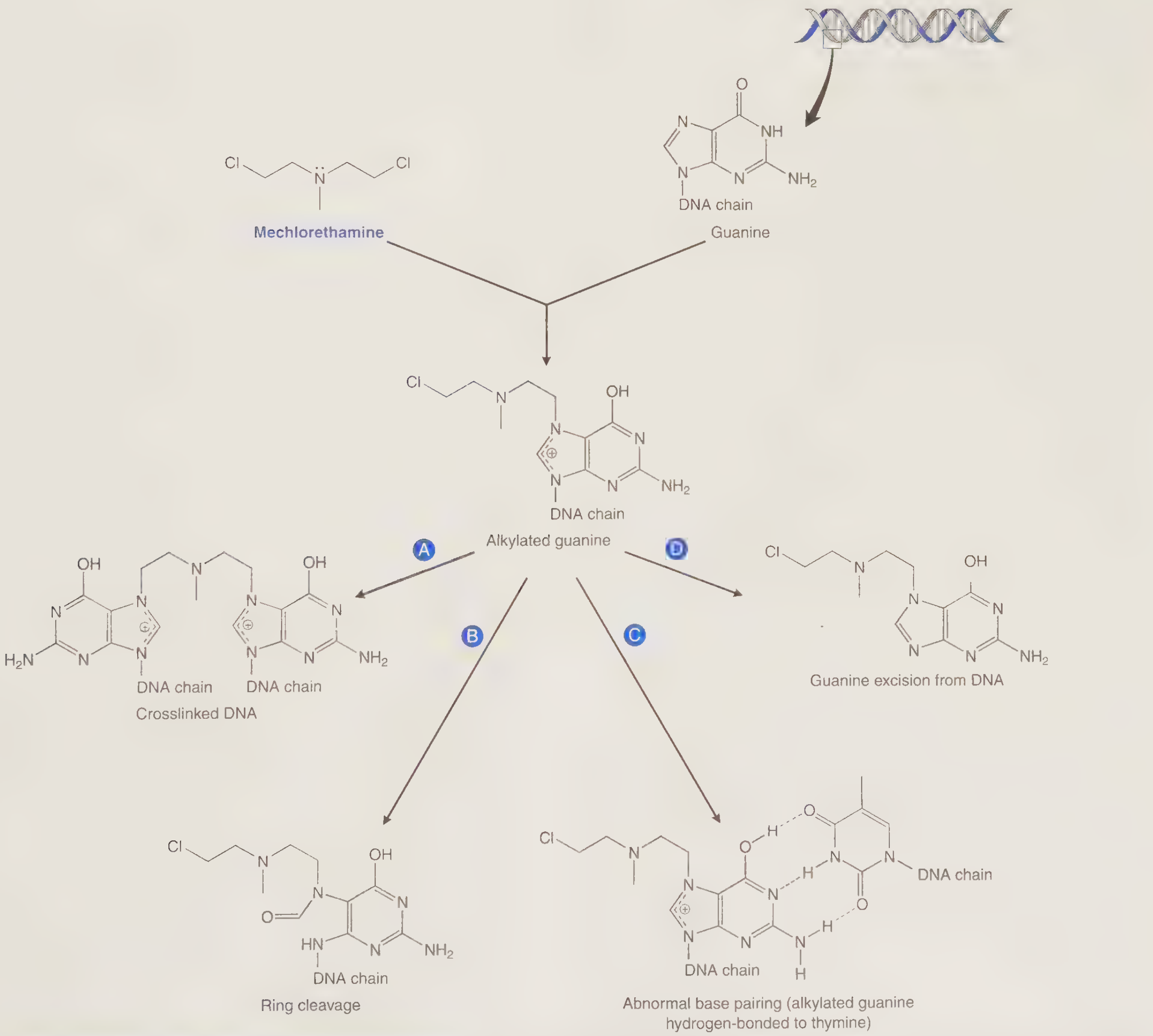

Mechlorethamine
Guanine
DNA chain
Alkylated guanine
A
B
C
D
Crosslinked DNA
Guanine excision from DNA
Ring cleavage
Abnormal base pairing (alkylated guanine hydrogen-bonded to thymine)

**Figure 32-9. Biochemical Outcomes of Guanine Alkylation.** In reactions such as those exemplified here with mechlorethamine, guanine alkylation can cause several types of DNA damage. The nitrogen of mechlorethamine performs a nucleophilic attack on one of its own β-carbons, resulting in an unstable intermediate that is highly electrophilic (not shown). The nucleophilic N-7 of guanine reacts with this unstable intermediate, resulting in an alkylated guanine. There are four potential outcomes that can result from this initial alkylation, all of which cause structural damage to DNA. **A.** The process of alkylation can be repeated, with a second guanine acting as a nucleophile. The resulting crosslinking of DNA seems to be a major mechanism by which alkylating agents damage DNA. **B.** Cleavage of the imidazole ring disrupts the structure of the guanine base. **C.** The alkylated guanine can hydrogen bond to thymine rather than cytosine, leading to a mutation in the DNA. **D.** Excision of the alkylated guanine residue results in a depurinated DNA strand.

lating agents can be exploited by infusing the drug directly into the site of a tumor. For example, thiotepa can be instilled into the bladder to treat superficial bladder cancers. In contrast to mechlorethamine and thiotepa, chlorambucil and melphalan are much less reactive, and can be administered orally. Cyclophosphamide is particularly useful because it is a nonreactive prodrug that requires activation by the hepatic cytochrome P450 system; this agent can be administered either orally or intravenously (Fig. 32-10).

**Nitrosoureas,** such as BCNU (**carmustine**), target DNA in much the same way as do cyclophosphamide and other alkylating agents. Like cyclophosphamide, these compounds require bioactivation. Unlike most alkylating agents, however, nitrosoureas also attach carbamoyl groups to their DNA-associated targets. It is not clear whether carbamoylation contributes significantly to the activity of nitrosoureas.

Some alkylating agents are better than others at targeting specific tumors. For example, nitrosoureas are useful in the treatment of brain tumors, because their high lipid solubility enables them to cross the blood–brain barrier. Similarly, the alkylating antibiotic **mitomycin** targets hypoxic tumor cells, such as those at the center of a solid tumor, because it requires bioreductive activation, which occurs more readily in low-oxygen settings.

Three other, nonclassical alkylating agents that are clinically useful deserve mention. The first is **dacarbazine,** a synthetic molecule that is a component of a potentially curative combination chemotherapy regimen for Hodgkin's disease. Dacarbazine also has some activity in treating melanoma and sarcomas. **Procarbazine** is an orally active drug that is used against Hodgkin's disease. A metabolite of procarbazine functions as a monoamine oxidase inhibitor, and toxicity related to this activity—such as tyramine sensitivity, hypotension, and dry mouth—can occur. Finally, **altretamine** is useful for treating refractory ovarian cancer. Although it is structurally related to alkylating agents of the triethylenemelamine class (such as thiotepa), whether or not the mechanism of action of this drug is related to DNA alkylation remains controversial.

Through natural selection, tumor cells can develop resistance to a single alkylating agent as well as cross-resistance to other drugs in the same class. Several mechanisms for resistance have been described. Highly reactive drugs can be deactivated by intracellular nucleophiles such as **glutathione.** Alternatively, cells can become resistant by reducing uptake of the drug or accelerating DNA repair. One enzyme, **$O^6$-alkylguanine-DNA alkyltransferase,** prevents permanent DNA damage by removing alkyl adducts that drugs add to the $O^6$ position of guanine before crosslinks are formed. Increased expression of this enzyme in neoplastic cells is associated with resistance to alkylating agents.

Alkylating agent toxicity is dose-dependent and can be severe. As a rule, toxic effects result from damage to DNA of normal cells. Three cell types are preferentially affected by alkylating agents. First, toxicity typically manifests in rapidly proliferating tissues, such as bone marrow, gastrointestinal and genitourinary tract epithelium, and hair follicles. This results in myelosuppression, gastrointestinal distress, and alopecia (hair loss). Second, organ-specific toxicity can result from low levels of a specific DNA damage repair enzyme in that tissue. Third, a tissue can be preferentially affected because the toxic compound accumulates in that tissue; for example, **acrolein** (a by-product of the activation of cyclophosphamide or its analogue **ifosfamide**) can produce hemorrhagic cystitis because of accumulation and concentration in the bladder. This toxicity can be treated by using the sulfhydryl-containing molecule **mesna,** which is also concentrated in the urine and rapidly inactivates the acrolein.

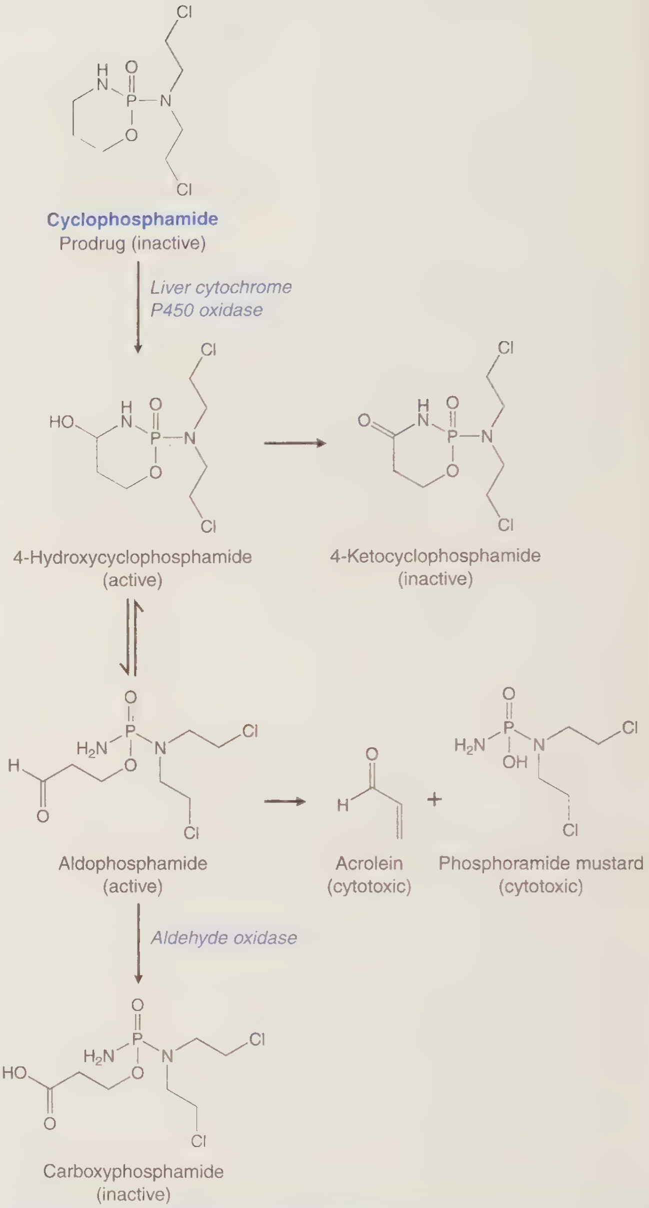

Cyclophosphamide
Prodrug (inactive)
Liver cytochrome P450 oxidase
4-Hydroxycyclophosphamide (active)
4-Ketocyclophosphamide (inactive)
Aldophosphamide (active)
Acrolein (cytotoxic)
Phosphoramide mustard (cytotoxic)
Aldehyde oxidase
Carboxyphosphamide (inactive)

**Figure 32-10. Activation and Metabolism of Cyclophosphamide.** Cyclophosphamide is a prodrug that must be oxidized by P450 enzymes in the liver to become pharmacologically active. Hydroxylation converts cyclophosphamide to 4-hydroxycyclophosphamide; this active metabolite can be further oxidized to the inactive metabolite 4-ketocyclophosphamide, or undergo ring cleavage to the active metabolite aldophosphamide. Aldophosphamide can be oxidized by aldehyde oxidase to the inactive metabolite carboxyphosphamide, or be converted to the highly toxic metabolites acrolein and phosphoramide mustard. Accumulation of acrolein in the bladder can cause hemorrhagic cystitis; this side effect of cyclophosphamide can be ameliorated by coadministration of mesna, a sulfhydryl compound that inactivates the acrolein (not shown).

The immune response requires rapid proliferation of lymphocytes; this makes lymphocytes especially vulnerable to damage by alkylating agents. Thus, in addition to their anticancer activity, alkylating agents such as cyclophosphamide are also effective at immunosuppression. This "toxicity" has been put to clinical use: when administered at doses lower than those needed for antineoplastic therapy, alkylating agents are used to treat autoimmune diseases and organ rejection (see Chapter 43, Pharmacology of Immunosuppression).

One approach to limiting toxicity has been to develop agents that accumulate preferentially inside tumor cells. An example of one such agent is melphalan, or phenylalanine mustard; this agent was designed to target melanoma cells, which accumulate phenylalanine for the biosynthesis of melanin. Another example is **estramustine,** in which the mustard component is conjugated to estrogen; this agent was designed to target breast cancer cells that express the estrogen receptor. Interestingly, neither melphalan nor estramustine works as intended, although they both have clinical utility; through mechanisms that are still poorly understood, melphalan is active against multiple myeloma and estramustine is used to treat prostate cancer.

## Platinum Compounds

The introduction of **cisplatin** (*cis*-diaminedichloroplatinum [II]) into clinical use in the 1970s transformed previously intractable tumors, such as testicular cancer, into curable ones. As with the alkylating agents, the anticancer properties of cisplatin were discovered by a chance observation. While studying the effects of electricity on bacteria, it was found that a product of the platinum electrode was inhibiting DNA synthesis in the microbes. The compound was purified and found to be cisplatin, which consists of a platinum atom bound to two amines and two chlorines in the *cis* conformation. This serendipitous finding led to the clinical use of cisplatin, which is now the most active drug used to treat testicular cancer (see the case of J.L., above). As an antitumor agent, cisplatin is thought to act similarly to *bis*-alkylating agents (i.e., alkylating agents with two leaving groups) by targeting nucleophilic centers in guanine, adenine, and cytosine.

The *cis* conformation of cisplatin (Fig. 32-11) allows the drug to form intrastrand crosslinks between adjacent guanine residues, resulting in DNA damage (Fig. 32-12B). This structural feature is critical to the action of cisplatin; the *trans* isomer, although capable of binding covalently to DNA, has little antitumor activity. Tumor cells can develop resistance to cisplatin by enhancing the repair of DNA lesions, decreasing drug uptake, or enhancing drug inactivation through upregulated synthesis of such nucleophiles as glutathione.

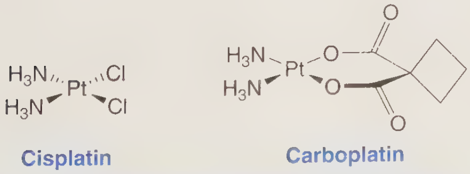


**Figure 32-11. Structures of Cisplatin and Carboplatin.** Cisplatin and carboplatin are coordinated complexes of platinum. The *cis* structure of these molecules (i.e., the presence of the two leaving groups on the same side of the molecule, rather than on opposite corners) provides them with the ability to crosslink adjacent guanines on the same DNA strand (intrastrand crosslink) or, much less frequently, on opposite DNA strands (interstrand crosslink). Similar compounds with *trans* conformations cannot effectively crosslink adjacent guanines.

As J.L.'s case demonstrates, cisplatin is efficacious in the treatment of genitourinary cancers, including cancers of the testis, bladder, and ovary. Cisplatin and the related compound carboplatin (Fig. 32-11) are also among the most efficacious drugs used against lung cancer. As with many chemotherapeutic agents, the rationale for the efficacy of cisplatin and carboplatin in the treatment of certain tumor types over others is not clear.

Cisplatin can be administered intravenously, but it can also be effective when exposed directly to tumor cells. One example is in treating ovarian cancer, which spreads along the inner lining of the peritoneal cavity. For this application, cisplatin can be infused directly into the peritoneal cavity to achieve high local concentrations of the drug while decreasing systemic toxicity.

J.L.'s oncologist considered cisplatin's toxicities carefully in determining the dose of this drug to administer and the other agents that would be included in the combination chemotherapy regimen. Because the dose-limiting toxicities of cisplatin, bleomycin, and etoposide differ from one another, each of these drugs could be used at the maximal tolerated dose (see Chapter 38, Principles of Combination Chemotherapy). For cisplatin, the dose-limiting toxicity is **nephrotoxicity.** Gastrointestinal symptoms such as nausea and vomiting are also common; this is of concern because dehydration with protracted vomiting can exacerbate cisplatin-induced kidney damage and lead to irreversible renal failure. Neurotoxicity, primarily manifested as paresthesias of the hands and feet, and hearing loss also occur frequently. Thiol-containing compounds, such as **amifostine,** can ameliorate cisplatin's nephrotoxicity without diminishing its antitumor effects. **Carboplatin,** a cisplatin analogue associated with much less nephrotoxicity, has replaced cisplatin in many chemotherapy regimens. **Oxaliplatin,** a third platinum compound, has recently been shown to have activity in the treatment of colorectal cancer. Like cisplatin, oxaliplatin causes cumulative neurotoxicity; oxaliplatin also induces a unique acute neurotoxicity that is exacerbated by exposure to cold temperatures.

## Bleomycin

The **bleomycins,** a family of glycopeptides synthesized by a species of *Streptomyces,* have prominent cytotoxic activity. A mixture of several of these glycopeptides, differing only in side chains, is used clinically (Fig. 32-12A). Bleomycin binds DNA and chelates iron (II), leading to the formation of free radicals that cause single- and double-stranded DNA breaks. As with many chemotherapeutic agents, multidrug resistance mechanisms, such as increased drug efflux from tumor cells, can reduce tumor susceptibility to bleomycin.

In chelating iron, bleomycin forms a heme-like ring. It is believed that the chelated complex abstracts a hydrogen radical from the 4′ position of a nearby pyrimidine residue (thymine or cytosine). The unstable intermediate decomposes in the presence of oxygen to produce an abstracted pyrimidine and a free phosphodiester at one or both DNA strands (Fig. 32-12A).

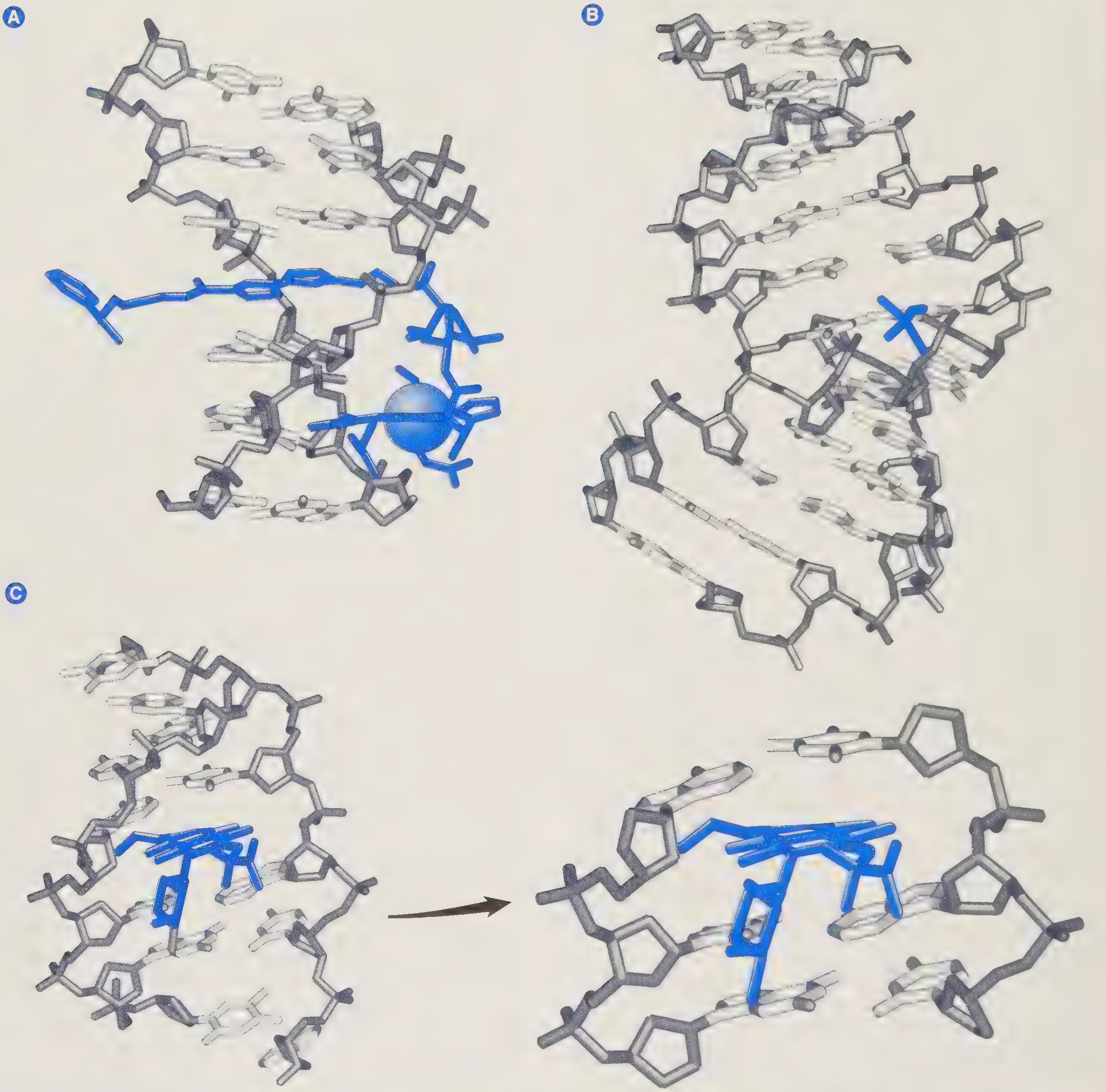


Figure 32-12. Interactions of Bleomycin, Platinum Compounds, and Anthracyclines with DNA. A. Bleomycin (highlighted in blue) binds to the DNA double helix and, thereby, exposes nucleotides in the DNA to the iron (II) atom (large blue ball) that is complexed to bleomycin. In the presence of molecular oxygen, the iron-bleomycin complex generates activated oxygen species that cause single-stranded and double-stranded breaks in the DNA by a free radical mechanism. B. Platinum complexes (highlighted in blue) crosslink N-7 atoms on adjacent guanine residues, forming intrastrand DNA crosslinks. C. Daunorubicin, an anthracycline (highlighted in blue), intercalates into the DNA structure (see expanded view on right) and, thereby, prevents the strand passage and religation steps that are part of the catalytic cycle of type II topoisomerase (see Fig. 32-5). Anthracyclines may also damage DNA by a free radical mechanism.

Relative to other DNA-damaging agents, bleomycin causes less myelosuppressive toxicity. Because of its reactivity with oxygen, however, bleomycin can cause **pulmonary fibrosis,** the drug's most problematic and dose-limiting toxicity. The effects of bleomycin on pulmonary function are cumulative and irreversible. Therefore, this agent's use is largely restricted to potentially curative combination chemotherapy regimens for testicular carcinoma and Hodgkin's disease. In J.L.'s case, it was concern regarding pulmonary toxicity that led his physician to monitor his lung function closely throughout therapy and to inquire about shortness of breath on each visit. Worsening lung function would have required adjustments in J.L.'s therapy.

## TOPOISOMERASE INHIBITORS

Several chemotherapeutic agents damage DNA by exploiting the natural nuclease/ligase function of topoisomerases (Figs. 32-4 and 32-5). The antineoplastic **camptothecins, anthracy-**

**clines, epipodophyllotoxins,** and **amsacrine,** and the antimicrobial **quinolones** act in this manner. These compounds interfere with the proper function of topoisomerases, and cause cellular topoisomerases to participate in DNA destruction.

## Camptothecins

The camptothecins are semisynthetic molecules derived from alkaloid extracts of *Camptotheca* plants. Camptothecins target topoisomerase I, causing DNA strand damage.

Topoisomerase I modulates supercoiling by complexing with DNA and nicking one of its two strands (Fig. 32-4). The camptothecins act by stabilizing this nicked DNA complex and preventing topoisomerase I from religating the strand break. Other replication enzymes then bind to the camptothecin–DNA–topoisomerase complex, resulting in a double-strand DNA break. Neoplastic cells are often unable to repair the resulting damage.

Two camptothecin derivatives, **irinotecan** and **topotecan,** have clinical significance. Irinotecan was initially introduced for the treatment of advanced colon cancer, although it may also be effective in treating other tumor types. It is a water-soluble prodrug that is cleaved by the enzyme carboxylesterase to release the lipophilic metabolite SN-38. Although SN-38 is approximately 1,000 times more active than irinotecan in inhibiting topoisomerase I, it is more highly protein-bound than irinotecan and has a much shorter half-life in vivo. Thus, the relative contribution of SN-38 to the anticancer effects of irinotecan is unclear. Irinotecan use is limited by severe gastrointestinal toxicity, leading to potentially life-threatening diarrhea. As with many other chemotherapeutic agents, irinotecan also causes dose-dependent bone marrow suppression.

Topotecan has been used in the treatment of metastatic ovarian cancer, small cell lung cancer, and other neoplasms. Specifically, this agent has shown effectiveness in treating ovarian neoplasms that are resistant to cisplatin, which are otherwise difficult to treat effectively.

## Anthracyclines

Anthracyclines, antitumor antibiotics isolated from a species of the fungus *Streptomyces,* are among the most clinically useful cytotoxic cancer chemotherapeutic agents. Although several mechanisms seem to be involved in their activity, the ability of the anthracyclines to damage DNA most likely results from their intercalation into DNA (Fig. 32-12C). This intercalation interferes with the action of topoisomerase II, resulting in such DNA lesions as strand scission and, ultimately, in cell death (Fig. 32-5).

As with many other antineoplastic agents, anthracyclines cause myelosuppression and alopecia. Anthracyclines are excreted in bile, and their dose must be reduced in patients with hepatic dysfunction. These agents are major components of chemotherapy regimens for a variety of malignancies, particularly hematologic cancers (such as leukemias and lymphomas) and breast cancer.

The best known drug in this group, **doxorubicin (Adriamycin),** is associated with **congestive heart failure.** It is thought that doxorubicin facilitates the excessive production of free radicals in the myocardium and, thereby, damages cardiac cell membranes. Cardiotoxicity is related to both the peak plasma concentration and the cumulative dose of doxorubicin. Cardiotoxicity can be reduced by coadministration of **dexrazoxane,** which is thought to inhibit free radical formation by chelating intracellular iron and preventing iron-mediated free radical generation.

## Epipodophyllotoxins

Like anthracyclines, **epipodophyllotoxins** seem to act primarily by inhibiting topoisomerase II-mediated religation of double-stranded DNA breaks (Fig. 32-5). The antineoplastic agents **etoposide (VP-16)** and **teniposide (VM-26)** are semisynthetic derivatives of a compound isolated from the plant *Podophyllum.* These drugs bind topoisomerase II and DNA, trapping the complex in its cleavable state. Tumor cells often develop resistance to etoposide by increasing their expression of **P-glycoprotein.** This protein normally serves as an efflux pump to rid the cell of toxic molecules such as natural metabolic side-products, but it can also remove chemotherapeutic agents derived from natural products before those agents have exerted their cytotoxic effect. Etoposide is useful for treating testicular cancer, lung cancer, and leukemia, while both etoposide and teniposide are used to treat various lymphomas.

Combining drugs that damage DNA directly, such as cisplatin and bleomycin, with drugs that inhibit topoisomerase II, such as etoposide, can have powerful synergistic anticancer effects. This synergy may relate to the role of topoisomerases in repairing DNA damage, or to the combined ability of these drug classes to induce sufficient DNA damage to trigger apoptosis. In practice, drugs of these classes are coadministered in many successful antineoplastic regimens. As J.L.'s case demonstrates, the combination of etoposide, bleomycin, and cisplatin can cure most cases of metastatic testicular cancer. Bone marrow suppression is the chief toxicity of the two epipodophyllotoxins in clinical use.

## Amsacrine

**Amsacrine** is another example of a chemotherapeutic agent that acts primarily by inhibiting topoisomerase II-mediated religation of double-stranded DNA breaks. This compound targets DNA by intercalating between base pairs, distorting the double helix, producing DNA-protein crosslinks, and creating both single- and double-stranded DNA lesions. Its clinical use is generally restricted to the treatment of recurrent leukemia and ovarian cancer.

## Antibacterial Topoisomerase Inhibitors: Quinolones

As noted above, not all classes of topoisomerase inhibitors are used against cancer. Inhibition of type II topoisomerase function is also the mechanism of action of the quinolones, a major class of bactericidal antibiotics that includes **ciprofloxacin, ofloxacin, levofloxacin,** and many other drugs identifiable by their generic names, which usually end in "–floxacin." Fluorinated quinolones (**fluoroquinolones**) are currently used to treat common urogenital, respiratory, and gastrointestinal infections caused by various Gram-negative microbes, including *Escherichia coli, Klebsiella pneumoniae, Campylobacter jejuni, Enterobacter, Pseudomonas aeruginosa, Neisseria gonorrhoeae,* and *Salmonella* and *Shigella* species. Bacteria typically evolve resistance through chromosomal mutations in the genes that encode type II topoisomerase or through alterations in the expression of membrane porins and efflux pumps that determine drug levels inside the bacteria. Adverse side effects of quinolone therapy are infrequent, but can include nausea, vomiting, and diarrhea.

Depending on the bacterial species being treated, quinolones act by inhibiting one of two prokaryotic type II topoisomerases, **DNA gyrase** (topoisomerase II) or **topoisomerase IV.** Selectivity of action against bacterial topoisomerases results from differences in structure between the prokaryotic and eukaryotic versions of these enzymes. Quinolones primarily inhibit DNA gyrase in Gram-negative organisms, and topoisomerase IV in Gram-positive organisms such as *Staphylococcus aureus.* Because resistant *S. aureus* is pervasive, the quinolones are less effective in treating infection caused by this bacterium. Thus, this class of drugs is used most frequently to treat Gram-negative organisms.

As is the case for chemotherapeutic drugs used to target eukaryotic tumor cell DNA, the mechanism of action of the quinolones involves subversion of the function of prokaryotic topoisomerase. Ordinarily, DNA gyrase binds to and nicks both strands of a DNA molecule, allowing another stretch of the same molecule to pass through and relieve supercoil strain (Fig. 32-5). Quinolones inhibit DNA gyrase before the second segment of DNA can pass through, thereby stabilizing the form of the complex in which the DNA polymer is broken. Although low doses of quinolones enable this inhibition to function reversibly, therapeutic doses stimulate dissociation of the DNA gyrase subunits, thereby releasing DNA with staggered double-stranded breaks and leading ultimately to cell death.

## MICROTUBULE INHIBITORS

Microtubules depend on dynamic instability for physiologic functioning. Without the ability to change length quickly, microtubules can do little other than lend structural support to a quiescent cell. Although microtubules play important roles in numerous aspects of cellular physiology, drugs that inhibit microtubule function are preferentially toxic to M-phase cells. Vinca alkaloids, colchicine, and griseofulvin inhibit microtubule polymerization, while taxanes inhibit microtubule depolymerization.

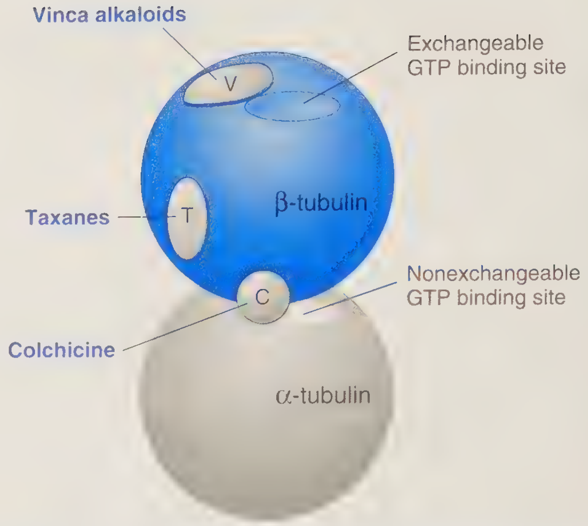

Vinca alkaloids
V
Exchangeable GTP binding site
Taxanes
T
β-tubulin
Nonexchangeable GTP binding site
C
Colchicine
α-tubulin

**Figure 32-13. Tubulin Binding Sites of Microtubule-Inhibiting Drugs.** The tubulin heterodimer is composed of α-tubulin (gray) and β-tubulin (blue). α-Tubulin and β-tubulin both bind GTP. The GTP on α-tubulin is not hydrolyzed; the GTP binding site on α-tubulin is, therefore, referred to as the nonexchangeable GTP binding site. β-Tubulin hydrolyzes GTP to GDP; the GTP binding site on β-tubulin is, therefore, referred to as the exchangeable GTP binding site. The three classes of microtubule inhibitors bind to distinct sites on the tubulin heterodimer. Vinca alkaloids, which inhibit microtubule polymerization, bind to a site on β-tubulin located near the exchangeable GTP binding site (V). Vinca alkaloids associate preferentially at the (+) end of microtubules and, thereby, inhibit the addition of new tubulin subunits to the microtubule. Colchicine, which also inhibits microtubule polymerization, binds to a site located at the interface between α-tubulin and β-tubulin (C). Colchicine alters the three-dimensional interactions between the tubulin subunits and, thereby, causes microtubule instability. Taxanes, which stabilize polymerized microtubules, bind to a site on β-tubulin (T). Taxanes may stabilize the interactions between tubulin subunits or, perhaps, stabilize the shape of microtubule protofilaments. Not shown is griseofulvin, an antifungal agent that may inhibit microtubule polymerization; its molecular interactions with tubulin are not well characterized.

## Inhibitors of Microtubule Polymerization

### *Vinca Alkaloids*

The vinca alkaloids **vinblastine** and **vincristine** are natural products originally isolated from the periwinkle plant, *Vinca rosea.* These agents inhibit tubulin polymerization and, thereby, prevent the extension of microtubules. Because microtubules must constantly add tubulin to maintain stability (i.e., they must retain a GTP-bound β-tubulin cap), inhibition of tubulin addition leads to the depolymerization of existing microtubules (Fig. 32-7).

Binding studies have suggested that vinca alkaloids bind to β-tubulin, on a portion of the molecule that overlaps with the GTP-binding domain (Fig. 32-13). When added to cells, vinca alkaloids are known to "poison" microtubules, resulting in microtubules that cannot extend. Experimental studies suggest that vinca alkaloid binding to the (+) end of microtubules inhibits the further addition of tubulin; this inhibition of tubulin addition to the (+) end of the microtubule eventually results in microtubule instability and depolymerization.

Vinblastine is used to treat certain lymphomas and, as part of a multidrug regimen (with cisplatin and bleomycin), to treat metastatic testicular cancer. Pharmacologic doses of the drug cause nausea and vomiting. **Myelosuppression** is the dose-limiting side effect of vinblastine.

Vincristine plays an important role in the chemotherapy of pediatric leukemias. It is also a component of chemotherapy regimens used to treat Hodgkin's disease and some non-Hodgkin's lymphomas. Pharmacologic doses of vincristine cause nausea and vomiting. Vincristine causes some myelosuppression, but not to the same degree as vinblastine. **Peripheral neuropathy** is usually the dose-limiting side effect of vincristine; this toxicity may result from the inhibition of microtubule function in long peripheral nerves that extend from the spinal cord to the extremities.

### Colchicine

**Colchicine** was known to inhibit mitosis long before it was recognized that the drug binds to tubulin. In fact, microtubules were first identified as a cellular component based on their ability to bind colchicine. As with the vinca alkaloids, colchicine inhibits microtubule polymerization by disrupting the three-dimensional structure of tubulin interactions. The colchicine-binding site is distinct from the vinca alkaloid site, and is found at the interface between α-tubulin and β-tubulin (Fig. 32-13). It has also been shown that the interaction of colchicine with microtubules occurs on the *interior* of the microtubule (recall that microtubules are hollow cylinders). Current theories suggest that colchicine binding to tubulin disrupts the interaction between α-tubulin and β-tubulin, either by bringing the two proteins closer together or pushing them further apart. Such an interaction likely destabilizes the stacking of tubulin subunits, leading to an unstable microtubule.

Colchicine is used in the treatment of acute attacks of gout (see Chapter 46, Integrative Inflammatory Pharmacology: Gout). Its mechanism of action in decreasing the pain of acute gout is unknown, but may be related to inhibition of protein trafficking, phagocytosis, and/or adhesion of neutrophils. Diarrhea is a common side effect of colchicine administration; this side effect follows from the fact that gastrointestinal mucosal cells replicate rapidly and are, therefore, highly sensitive to an inhibitor of mitosis.

### Griseofulvin

**Griseofulvin** is an uncommonly prescribed drug that acts by inhibiting the assembly of microtubules and the activity of accessory proteins necessary for mitotic spindle formation. Its exact mechanism of action is not well characterized. Following absorption from the GI tract, griseofulvin concentrates in the keratinized layers of the skin; this property makes the drug useful against dermatophyte mycoses. Other antifungal agents are discussed in Chapter 36, Pharmacology of Fungal Infections.

## Inhibitors of Microtubule Depolymerization: Taxanes

The taxanes, which include **paclitaxel** and **docetaxel**, are natural products originally derived from the bark of the western yew tree. Taxanes bind to the β-tubulin subunit of microtubules at a site distinct from the vinca alkaloid and colchicine binding sites (Fig. 32-13). As with colchicine, paclitaxel has been shown to bind to the *inside* of microtubules. Unlike the other agents, however, taxanes promote microtubule polymerization and, conversely, inhibit depolymerization. Stabilization of the microtubules in a polymerized state arrests cells in mitosis and, eventually, leads to the activation of apoptosis.

There are two hypotheses for the apparent microtubule-stabilizing properties of taxanes. First, taxanes could strengthen the lateral interactions between microtubule protofilaments. Increased lateral interactions would decrease the tendency for protofilaments to "peel" away from the microtubule cylinder. Second, taxanes could straighten individual protofilaments. Once β-tubulin hydrolyzes GTP to GDP, protofilaments have a tendency to "curl," which produces a strain on the integrity of the microtubule cylinder. By straightening protofilaments, taxanes could reduce the tendency for the protofilaments to separate from the intact microtubule. In vivo, both of these mechanisms may be important for taxane-mediated stabilization of microtubules; alternative mechanisms are also possible.

Paclitaxel is used as an antineoplastic agent in the treatment of many solid tumors, especially breast, ovarian, and non–small cell lung cancer. Paclitaxel has a number of important side effects. An acute hypersensitivity reaction occurs commonly in response to paclitaxel, or more likely to the vehicle in which paclitaxel is solubilized; this effect can be obviated by administration of dexamethasone and a histamine H1 receptor antagonist before treatment with paclitaxel. Many patients experience myalgias and myelosuppression from paclitaxel, and high doses of this drug can cause pulmonary toxicity. **Peripheral neuropathy,** usually manifesting as a stocking-glove sensory deficit in the extremities, can limit the cumulative amount of drug that can be safely administered.

Docetaxel has found recent application in breast cancer and non–small cell lung cancer. As with paclitaxel, docetaxel causes an acute hypersensitivity reaction that can be obviated by preadministration of glucocorticoids. Docetaxel occasionally exhibits the drug-specific side effect of fluid retention, which likely arises from increased capillary permeability. Docetaxel does not cause neuropathy as frequently as paclitaxel. The myelosuppression associated with docetaxel is profound, however, and is usually dose-limiting.

## ■ *Conclusion and Future Directions*

Inhibitors of DNA replication and mitosis are critical components of current treatment strategies directed against a number of cancers and bacterial infections. Some of these agents are also used to induce immune suppression (e.g., cyclophosphamide). Proper replication and transcription of DNA require the presence of an intact sequence of deoxyribonucleotides and the normal functioning of topoisomerases, which control DNA supercoiling. The various classes of DNA damaging drugs demonstrate different mechanisms of action. These include alkylation (alkylating agents) or carbamoylation (nitrosoureas), generation of crosslinks (carboplatin and cisplatin), formation of oxidative lesions (bleomycin), and inhibition of topoisomerase function (camptothecins, anthracyclines, epipodophyllotoxins, amsacrine, and quinolones). Because DNA integrity is critical for cell viability, DNA-damaging agents are cytotoxic and bactericidal. Microtubule inhibitors interrupt the cell cycle during mitosis and are also cytotoxic. The toxicities of some of these agents are severe, but coadministration of protective compounds can, in some cases, limit these toxic effects.

In an effort to increase the utility of antineoplastic DNA damaging agents, current research is proceeding along several lines. These include targeting agents directly to tumors; developing systems in which agents are activated selectively within tumor cells; and combining agents with compounds that increase their intracellular concentration, block DNA repair, or lower a cell's threshold for undergoing apoptosis. The goal of these approaches is to increase the therapeutic index of the drugs, allowing for more successful treatment of cancer with less toxicity.

## Suggested Readings

Abang AM. The clinical pharmacology of topoisomerase I inhibitors. Semin Hematol 1998;35:13–21. (*Clinical trials provide data for this review of the pharmacology of camptothecin congeners, including topotecan, irinotecan, and the biologically active irinotecan metabolite SN-38.*)

Alberts B, et al. Molecular Biology of the Cell. 4th Ed. New York: Garland Publishing, Inc., 2002. (*Excellent background for basic physiology of DNA structure, DNA replication, and mitosis.*)

Fleming RA. An overview of cyclophosphamide and ifosfamide pharmacology. Pharmacotherapy 1997;17:146S–154S. (*Discusses the DNA-damaging mechanisms, current understanding of tumor resistance, and toxicities of these alkylating agents.*)

Lawley PD, Phillips DH. DNA adducts from chemotherapeutic agents. Mutat Res 1996;355:13–40. (*Thorough review of DNA damage caused by alkylation.*)

Muller I, Niethammer D, Bruchelt G. Anthracycline-derived chemotherapeutics in apoptosis and free radical cytotoxicity. Interl J Molec Med 1998;1:491–494. (*Reviews the mechanism of action of representative anthracyclines, and discusses the oxidative stress and relevant cell biology governing the dose-limiting cardiomyopathy that complicates anthracycline treatment.*)

O'Donnell JA, Gelone SP. Fluoroquinolones. Infect Dis Clin N Am 2000;14:489–513 (*Discusses the mechanism of action of fluoroquinolone antibiotics.*)

Trimmer EE, Essigmann JM. Cisplatin. Essays Biochem 1999;34:191–211. (*Overview of mechanisms for cisplatin action and drug resistance.*)

## Drug Summary Table Pharmacology of DNA Replication and Mitosis

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **AGENTS THAT DIRECTLY MODIFY DNA STRUCTURE**<br>*Mechanism—See Notes* | | | | |
| **Alkylating agents**<br>**Cyclophosphamide**<br>**Mechlorethamine**<br>**Melphalan**<br>**Estramustine**<br>**Chlorambucil**<br>**Mitomycin**<br>**Triethyleneamine**<br>**Thiotepa**<br>**Nitrosoureas**<br>**Carmustine**<br>**Atypicals**<br>**Dacarbazine**<br>**Procarbazine**<br>**Altretamine** | Cyclophosphamide: immune suppression, lymphoma<br>Melphalan: lymphoma<br>Estramustine: prostate cancer<br>Thiotepa: bladder cancer<br>Nitrosoureas: brain cancer<br>Mitomycin: reductive activation favors use in anoxic tumor cores<br>Dacarbazine, procarbazine: Hodgkin's disease<br>Altretamine: refractory ovarian cancer | All alkylating agents can cause bone marrow, gastrointestinal and genitourinary tract epithelium, hair follicle toxicity<br>Cyclophosphamide (→ acrolein): hemorrhagic cystitis; prevent with mesna<br>Mechlorethamine: vesicant; administer by injection | Pregnancy (relative) | Covalently bind DNA, often crosslink to DNA or associated protein, leading to apoptosis<br>Cyclophosphamide, nitrosoureas, and mitomycin are prodrugs |
| **Platinum compounds**<br>**Cisplatin**<br>**Carboplatin**<br>**Oxaliplatin** | Cisplatin and carboplatin: genitourinary (testicular, bladder, ovarian) and lung cancer<br>Oxaliplatin: colorectal cancer | Cisplatin: nephrotoxicity; prevent with amifostine<br>Oxaliplatin: acute neurotoxicity | Pregnancy (relative)<br>Cisplatin and carboplatin: synergy with etoposide<br>Oxaliplatin: synergy with 5-fluorouracil | Crosslink guanines (intrastrand)<br>Carboplatin is less toxic than cisplatin<br>Oxaliplatin neurotoxicity is cold-sensitive |
| **Bleomycin** | Testicular cancer<br>Hodgkin's disease | Pulmonary fibrosis | Synergy with etoposide<br>Preexisting severe pulmonary disease<br>Pregnancy (relative) | Binds $O_2$ and chelates Fe(II), binds DNA and leads to strand breaks via oxidative intermediates; specific to G2 |
| **TOPOISOMERASE INHIBITORS**<br>*Mechanism—Inhibit topoisomerases, leading to DNA strand breakage* | | | | |
| **Camptothecins**<br>**Irinotecan**<br>**Topotecan** | Irinotecan: colon cancer<br>Topotecan: ovarian and small cell lung cancer, esp. cisplatin-resistant cancer | Gastrointestinal toxicity leading to life-threatening diarrhea, myelosuppression | Pregnancy (relative) | Inhibit topoisomerase I<br>Derivative formed by carboxylesterase cleavage may also have activity<br>Specific to S phase |
| **Anthracyclines**<br>**Doxorubicin (Adriamycin)**<br>**Daunorubicin**<br>**Epirubicin** | Leukemias and lymphomas, breast cancer | Myelosuppression, alopecia<br>Doxorubicin: CHF (prevent with dexrazoxane) | Congestive heart failure<br>Pregnancy (relative) | Inhibit topoisomerase II<br>Excreted in bile (reduce dose in patients with hepatic dysfunction)<br>Intercalate into DNA<br>Specific to G2 |
| **Epipodophyllotoxins**<br>**Etoposide**<br>**Teniposide** | Etoposide: testicular and lung cancer, leukemia, lymphoma<br>Teniposide: lymphoma | | Pregnancy (relative) | Inhibit topoisomerase II<br>Synergy with cisplatin, bleomycin<br>Act in late S to G2 |

*(Continued)*

## Drug Summary Table Pharmacology of DNA Replication and Mitosis *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **TOPOISOMERASE INHIBITORS** *(Continued)*<br>***Mechanism—Inhibit topoisomerases, leading to DNA strand breakage*** | | | | |
| Amsacrine | Recurrent leukemias, ovarian cancer | | | Inhibits topoisomerase II |
| Quinolones<br>**Ciprofloxacin**<br>**Ofloxacin**<br>**Levofloxacin** | *Streptococcus, Staphylococcus, Haemophilus, Legionella, Chlamydia, Mycoplasma, Moraxella, Bacillus, E. coli, Klebsiella, Campylobacter, Enterobacter, Pseudomonas, Neisseria, Salmonella,* and *Shigella* species | | | Bactericidal<br>Inhibit topoisomerase II (gyrase) in Gram-negative organisms, topoisomerase IV in Gram-positive organisms |
| **AGENTS THAT INHIBIT MICROTUBULE POLYMERIZATION**<br>***Mechanism—Bind tubulin subunits and prevent microtubule polymerization*** | | | | |
| Vinca alkaloids | | | | |
| **Vinblastine** | Metastatic testicular cancer<br>Lymphomas | Nausea, vomiting<br>Bone marrow suppression (dose-limiting) | IV use only<br>Pregnancy (relative) | |
| **Vincristine** | Pediatric leukemias<br>Hodgkin's disease<br>Non-Hodgkin's lymphoma | Nausea, vomiting<br>Peripheral neuropathy (dose-limiting) | IV use only<br>Pregnancy (relative) | |
| **Colchicine** | Acute gout | Diarrhea | ↑ levels with cyclosporine, verapamil | Inhibits microtubule assembly |
| **Griseofulvin** | Tinea of skin, hair and nails | | Porphyria<br>Liver disease | Concentrates in keratinized layers of the skin |
| **AGENTS THAT INHIBIT MICROTUBULE DEPOLYMERIZATION**<br>***Mechanism—Bind polymerized tubulin and prevent microtubule depolymerization*** | | | | |
| **Taxanes** | | | | |
| **Paclitaxel** | Ovarian cancer<br>Breast cancer<br>Lung cancer (non-small cell) | Hypersensitivity reaction<br>Myopathy<br>Neuropathy (dose-limiting)<br>Myelosuppression<br>Pulmonary toxicity (high-doses) | Pregnancy (relative) | Dexamethasone and histamine $H_1$ antagonist prior to treatment reduces hypersensitivity |
| **Docetaxel** | Breast cancer<br>Lung cancer (non-small cell) | Hypersensitivity reaction<br>Fluid retention syndrome<br>Myelosuppression (dose-limiting)<br>Neuropathy | Pregnancy (relative) | Neuropathy less frequent than with paclitaxel<br>Dexamethasone prior to treatment reduces hypersensitivity and fluid retention syndrome |

# 33

# Pharmacology of Transcription and Translation

Marvin Ryou and Donald M. Coen

Introduction
Case
Biochemistry of Prokaryotic Transcription and Translation
Pharmacologic Classes and Agents
- Inhibitors of Transcription
- Inhibitors of Translation
  - *Antimicrobial Drugs Targeting the 30S Subunit*
    - *Aminoglycosides*
    - *Spectinomycin*
    - *Tetracyclines*
  - *Antimicrobial Drugs Targeting the 50S Subunit*
    - *Macrolides*
    - *Chloramphenicol*
    - *Lincosamides*
    - *Streptogramins*
    - *Oxazolidinones*

Conclusion and Future Directions
Suggested Readings

## INTRODUCTION

Fundamental biochemical differences between bacteria and humans are exploited for the development and clinical use of antibiotics. Two such differences that are targeted by antibacterial chemotherapeutic drugs involve the molecular and cellular machinery of transcription and translation. At the level of transcription, drugs such as rifampin (a mainstay in the therapy of tuberculosis) bind to and inhibit bacterial RNA polymerase. At the level of translation, a plethora of drugs bind bacterial ribosomes to inhibit protein synthesis. Specifically, aminoglycosides, tetracyclines, and spectinomycin bind the 30S ribosomal subunit, while macrolides, chloramphenicol, lincosamides, streptogramins, and oxazolidinones target the 50S ribosomal subunit. These inhibitors of protein synthesis generally act on both Gram-positive and Gram-negative organisms and, therefore, are in wide clinical use (see Chapter 35 for a discussion of Gram-positive and Gram-negative bacteria). This chapter briefly reviews the biochemistry of transcription and translation in prokaryotes, and discusses the major differences between these processes in prokaryotes and eukaryotes. The biochemical basis of transcription and translation is then used to discuss the mechanisms by which various pharmacologic inhibitors block these processes.

## Case

It is the summer of 1976. Participants returning from an American Legion convention in Philadelphia are falling severely ill with a mysterious type of pneumonia. The outbreak centers on the Bellevue Stratford Hotel, where 150 hotel occupants and 32 passers-by contract "Legionnaires' Disease." Twenty-nine victims ultimately die. Conventional sputum stains, cultures, and even autopsy material show no consistent pathogens. The terror of an unknown epidemic disease sparks rumors and news reports of poison gases, tainted water supplies, terrorists, and deadly viruses.

Several months later, laboratory and field investigation teams from the Centers for Disease Control and Prevention (CDC) identify the causative aerobic Gram-negative bacterium, and name it *Legionella pneumophila*. It is observed that cases treated with erythromycin and tetracycline have better outcomes than those treated with other agents. Today, erythromycin and the other macrolides, clarithromycin and azithromycin, are the drugs of choice for treating Legionnaires' Disease—as well as many chlamydial, streptococcal, and staphylococcal infections.

## QUESTIONS

1. Which steps in translation are blocked by tetracyclines and macrolides?
2. How do bacteria develop resistance to these drugs and to other inhibitors of transcription and translation?
3. Why are macrolides bacteriostatic, while some antibiotics, such as aminoglycosides, are bactericidal?
4. Why are macrolides the most effective treatment for Legionnaires' Disease?

## BIOCHEMISTRY OF PROKARYOTIC TRANSCRIPTION AND TRANSLATION

It is important to note that the following discussion of prokaryotic gene expression is simplified to emphasize the steps that are inhibited by antibiotics.

Gene expression begins with a process called transcription, which involves the synthesis of single-stranded RNA transcripts from a DNA template. Transcription is catalyzed by the enzyme **RNA polymerase.** In bacteria, five subunits (2 $\alpha$, 1 $\beta$, 1 $\beta'$, and 1 $\sigma$) associate to form the holoenzyme. As discussed below, the $\sigma$ subunit is instrumental for initiating transcription, while the rest of the RNA polymerase enzyme—otherwise known as the core enzyme—contains the catalytic site for RNA synthesis.

*The process of transcription occurs in three stages: initiation, elongation, and termination* (Fig. 33-1). During initiation, the RNA polymerase holoenzyme separates the strands of a short segment of double-helical DNA after its $\sigma$ subunit recognizes an upstream site. Once the double helix is unwound to form a single-stranded template, RNA polymerase initiates RNA synthesis at a start site on the DNA. During elongation, RNA polymerase synthesizes a complementary RNA strand by joining together ribonucleoside triphosphates via phosphodiester bonds. In the process, the $\sigma$ subunit dissociates from the holoenzyme. RNA synthesis proceeds in the $5' \rightarrow 3'$ direction until a termination sequence is reached.

The RNA polymerase enzyme differs between bacteria and humans and, thus, can serve as a selective target for antibacterial drug action. In bacteria, one RNA polymerase synthesizes all of the RNA in the cell (except the short RNA primers needed for DNA replication, which are made by **primase**). Furthermore, bacterial RNA polymerase is composed of only 5 subunits. In contrast, eukaryotes express three different RNA polymerases, and each enzyme is considerably more complex in its subunit structure than the bacterial counterpart. For example, the type II eukaryotic RNA polymerase, which synthesizes the precursors of mRNA, consists of 8–12 subunits. The process of transcription, as in that of DNA replication, also depends on **DNA topoisomerases** to regulate the supercoiling of DNA. Drugs that target bacterial topoisomerases are discussed in Chapter 32, Pharmacology of DNA Replication and Mitosis.

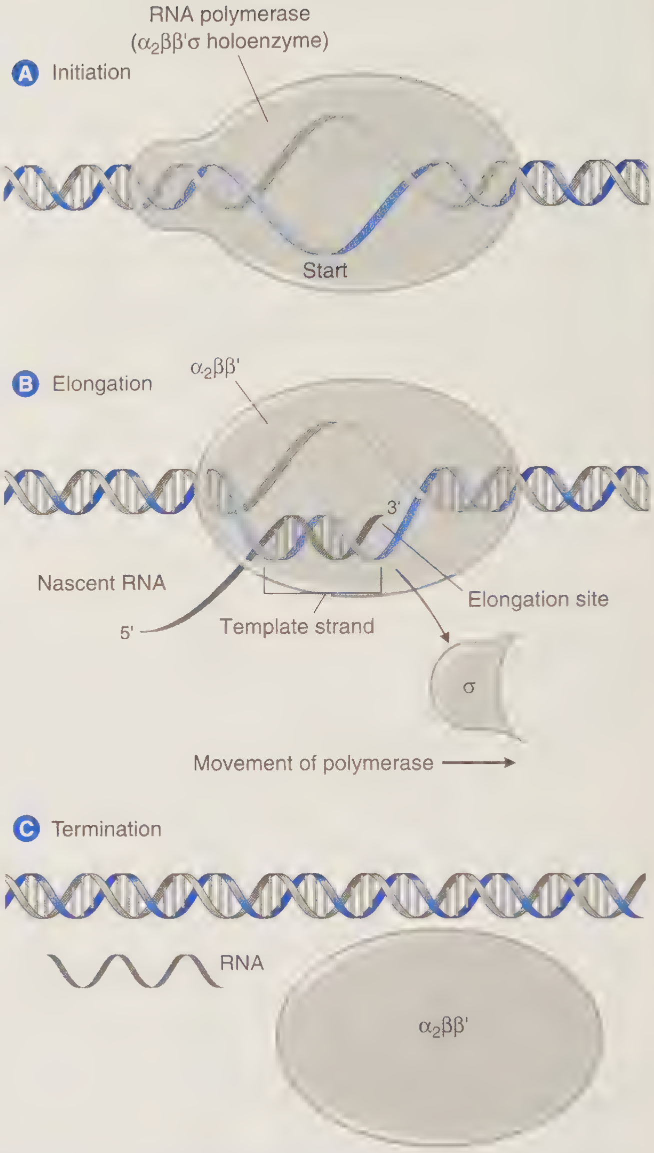


**Figure 33-1. Prokaryotic Transcription. A.** During initiation, the RNA polymerase holoenzyme ($\alpha_2\beta\beta'\sigma$) searches for and recognizes promoter sequences on DNA. The holoenzyme then separates the strands of the double helical DNA, exposing the start site for transcription. **B.** During elongation, the core enzyme (without the $\sigma$ subunit) synthesizes the new RNA strand in the $5' \rightarrow 3'$ direction, using the unwound DNA strand as a template. RNA polymerase separates the strands of the DNA double helix as it moves along the template strand, extruding the 5′ end of the transcript behind it. Rifampin blocks elongation by complexing with the $\beta$ subunit of RNA polymerase (not shown). **C.** Upon reaching a termination sequence, the DNA, core enzyme, and newly synthesized RNA separate from one another.

Once the messenger RNA (mRNA) transcripts are synthesized, the transcripts are translated by the bacterial translational machinery. Although the overall process of translation is similar between bacteria and higher organisms, there are a number of pharmacologically exploitable differences in the details of the mechanisms. In particular, the number and composition of the ribosomal RNA (rRNA) molecules differ between bacterial and human ribosomes. Thus, bacterial ribosomes can also serve as targets for selective antibiotics.

The ribosome of the representative bacterium, *E. coli,* has a sedimentation coefficient of **70S** and is composed of a **30S**

**subunit** and a **50S subunit.** The 30S subunit contains a single **16S rRNA** molecule and 21 different proteins, while the 50S subunit contains two rRNA molecules—**23S rRNA** and **5S rRNA**—and 31 different proteins. Importantly, it is the rRNA rather than the protein components of the ribosome that are responsible for the ribosome's key activities; namely, decoding the mRNA, linking together amino acids, and translocating the translation machine. The 70S ribosome contains two sites that are critical for translation: the **P** or "**peptidyl**" **site,** which contains the growing peptide chain, and the **A** or "**aminoacyl**" **site** (also known as the "**acceptor**" **site**), which binds incoming tRNA molecules carrying the various amino acids (Fig. 33-2).

Translation, like transcription, can be divided into three steps (Fig. 33-3). During **initiation,** the components of the translation system assemble together. First, the mRNA joins with the 30S subunit of the bacterial ribosome and with a specific tRNA molecule linked to **formylated methionine** (fMet), the first amino acid encoded by every bacterial mRNA. The tRNA-formylated methionine molecule (fMet-tRNA$_f$) binds to its initiation codon (AUG) on the mRNA. Next, the 50S

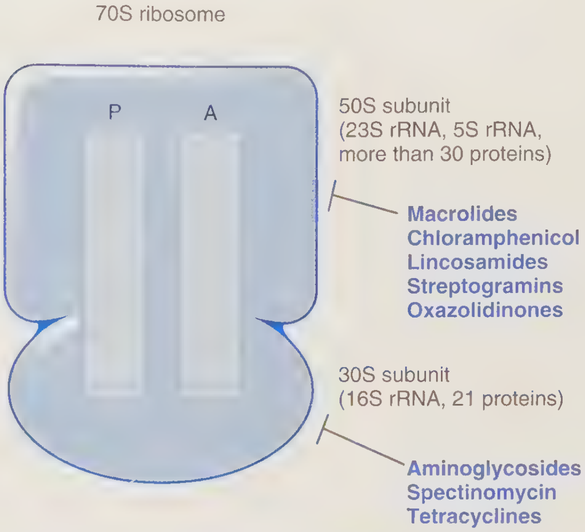

70S ribosome
P
A
50S subunit
(23S rRNA, 5S rRNA,
more than 30 proteins)
Macrolides
Chloramphenicol
Lincosamides
Streptogramins
Oxazolidinones
30S subunit
(16S rRNA, 21 proteins)
Aminoglycosides
Spectinomycin
Tetracyclines

**Figure 33-2. The Prokaryotic 70S Ribosome.** The prokaryotic 70S ribosome consists of a 30S subunit and a 50S subunit. Each subunit is composed of ribosomal RNA (rRNA) and numerous proteins. The rRNAs are responsible for most of the important activities of the ribosome and are the targets of antibiotic drugs that inhibit translation. Aminoglycosides, spectinomycin, and tetracyclines bind to and inhibit the activity of 16S rRNA in the 30S subunit. Macrolides, chloramphenicol, lincosamides, streptogramins, and oxazolidinones bind to and inhibit the activity of 23S rRNA in the 50S subunit. A, aminoacyl site (site of binding of aminoacyl tRNA). P, peptidyl site (site of binding of tRNA that is covalently joined to the elongating peptide chain).

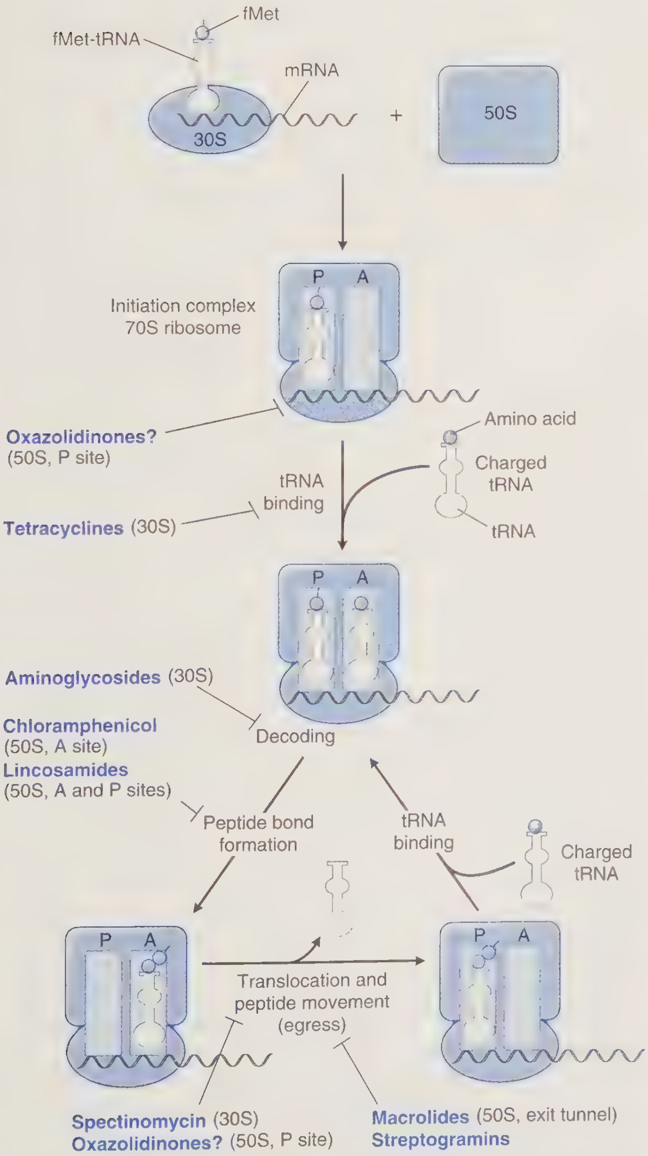

fMet-tRNA
fMet
mRNA
30S
+
50S
Initiation complex
70S ribosome
P
A
Oxazolidinones?
(50S, P site)
Amino acid
Charged
tRNA
tRNA
binding
Tetracyclines (30S)
tRNA
P
A
Aminoglycosides (30S)
Chloramphenicol
(50S, A site)
Lincosamides
(50S, A and P sites)
Decoding
Peptide bond
formation
tRNA
binding
Charged
tRNA
P
A
Translocation and
peptide movement
(egress)
P
A
Spectinomycin (30S)
Oxazolidinones? (50S, P site)
Macrolides (50S, exit tunnel)
Streptogramins

**Figure 33-3. Prokaryotic Translation.** Prokaryotic translation begins with the assembly of a complex containing a 30S ribosomal subunit, mRNA, formyl-methionine-linked tRNA (fMet-tRNA), and a 50S ribosomal subunit. This assembly step is dependent on the binding of fMet-tRNA to an initiator codon in the mRNA. The assembled 70S ribosome contains two binding sites, referred to as the aminoacyl (A) and peptidyl (P) sites. The A site accepts incoming triplet codons of mRNA and allows the corresponding amino acid-linked tRNA (i.e., charged tRNA) to bind to its corresponding triplet. The decoding function of 16S rRNA helps ensure that the mRNA codon binds to the correct tRNA. Once a charged tRNA has entered the A site, the peptidyl transferase activity of the 23S rRNA catalyzes the formation of a peptide bond between the amino acid occupying the A site and the carboxy-terminus of the nascent peptide residing in the P site. Once the peptide bond has formed, the tRNA–mRNA complex translocates from the A site to the P site, the tRNA molecule that had occupied the P site dissociates from the P site, and the elongating polypeptide chain moves out through the exit tunnel. The A site is now empty, and introduction of the next charged tRNA molecule into the A site completes the cycle. Translation continues until a stop codon is encountered in the mRNA, at which point the newly synthesized protein is released from the ribosome.

Pharmacologic agents that inhibit translation interfere with the activities of the prokaryotic ribosome. Aminoglycosides bind to rRNA in the 30S subunit and enable the binding of incorrect tRNAs to mRNA; tetracyclines block aminoacyl-tRNA binding to the A site; chloramphenicol and lincosamides inhibit the peptidyl transferase activity of the 50S subunit. Spectinomycin, macrolides, and streptogramins inhibit peptide translocation. The mechanism(s) of action of the oxazolidinones are uncertain, but some possible sites of action are indicated.

subunit joins with the 30S subunit to form the complete 70S ribosome. The fMet-tRNA$_f$ now occupies the P site of the 70S ribosome.

**Elongation** involves the addition of amino acids to the carboxyl end of the growing polypeptide chain, as the ribosome moves from the 5′-end to the 3′-end of the mRNA that is being translated. tRNA molecules carrying specific amino acids (aminoacyl tRNAs) enter the ribosomal A site and base-pair to their proper codons on the mRNA. Utilization of the correct tRNA requires not only anticodon–codon recognition between tRNA and mRNA, respectively, but also **decoding** functions provided by the 16S rRNA in the 30S ribosomal subunit. **Peptidyl transferase,** an enzyme whose activity derives from the 23S rRNA of the 50S subunit (i.e., peptidyl transferase is a ribozyme), catalyzes the formation of a peptide bond between fMet and the next amino acid. Formation of the peptide bond causes fMet to be linked to the next amino acid, which, in turn, is linked to the tRNA in the A site (i.e., the tRNA in the A site has "accepted" the fMet). After the peptide bond has been formed, the ribosome advances three nucleotides toward the 3′-end of the mRNA. In the process, the tRNA$_f$ that was originally linked to the fMet is ejected from the P site, the tRNA that is now linked to two amino acids shifts from the A site to the unoccupied P site, the A site becomes available, and the growing peptide emerges from the exit tunnel of the ribosome. This process is known as **translocation.** In this manner, polypeptide chain elongation results from multiple cycles of aminoacyl tRNA binding to the A site, peptide bond formation, and translocation.

During **termination,** proteins called **release factors** recognize the termination codon in the A site, and activate discharge of the newly synthesized protein and dissociation of the ribosome-mRNA complex. This process seems to involve, at least in some cases, structural mimicry of tRNAs by the release factors.

Three general points are worth noting about bacterial translation. First, *the two ribosomal subunits demonstrate segregated functions:* the 30S subunit is responsible for faithful decoding of the mRNA message, while the 50S subunit catalyzes peptide bond formation. Translocation, however, seems to involve both subunits. Second, *the catalytic machinery resides in the RNA component of the ribosome, not in the ribosomal proteins.* In other words, it is the rRNA that "does the work." Third, *inhibitors of protein synthesis block the process of translation at different steps.* Experimentally, the field of bacterial genetics has played a pivotal role in deducing specific drug targets via analyses of drug-resistant mutants. More recent work, using nuclear magnetic resonance spectroscopy and x-ray crystallography, has further elucidated the structures of bacterial RNA polymerase and bacterial ribosomes as well as the molecular nature of the various drug-target interactions.

## PHARMACOLOGIC CLASSES AND AGENTS

### INHIBITORS OF TRANSCRIPTION

**Rifampin** and its structural relative, **rifabutin,** are two semisynthetic derivatives of the naturally occurring antibiotic **rifamycin B.** Rifampin can be used for prophylaxis of meningococcal disease, but it is currently used almost exclusively in the treatment of tuberculosis and other mycobacterial infections that are a growing problem in patients with AIDS. Rifampin is particularly effective against phagosome-dwelling mycobacteria because it is bactericidal for intracellular as well as extracellular bacteria. Furthermore, rifampin increases the in vitro activity of isoniazid, another front-line drug used in the combination therapy of tuberculosis (see Chapter 35, Pharmacology of the Bacterial Cell Wall, and Chapter 38, Principles of Combination Chemotherapy).

Rifampin exerts its bactericidal activity by forming a stable complex with bacterial DNA-dependent RNA polymerase, thereby inhibiting RNA synthesis. The drug targets the β subunit of bacterial RNA polymerase; it permits initiation, but then blocks the path of elongating RNA once it becomes 2–3 nucleotides in length. Rifampin displays high specificity for bacteria, as mammalian polymerases (even those of mitochondria, which are considered prokaryote-like) are inhibited by rifampin only at far higher concentrations. Hence, rifampin is generally well tolerated, and the incidence of side effects (usually rash, fever, nausea, vomiting, and jaundice) is low.

Because rapidly emerging resistance makes single drug therapy of tuberculosis not only ineffective but also counterproductive, rifampin is administered in combination with other antituberculosis drugs. In vitro experiments show that one out of every $10^7$–$10^8$ tubercle bacilli can develop resistance to rifampin via a one-step mutational process within the drug target site. However, as a part of a multidrug therapeutic regimen, rifampin can markedly reduce the lifetime rate of reactivation of latent tuberculosis (see Chapter 38).

### INHIBITORS OF TRANSLATION

Three general considerations apply to inhibitors of translation. First, *translational inhibitors target either the 30S or 50S subunits of the bacterial ribosome.* Although the details can be confusing, as in the case of the new oxazolidinone class of drugs, the following discussion of translational inhibitors is presented in terms of 30S versus 50S inhibition (Table 33-1).

The second consideration concerns selectivity. *In addition to their inhibitory effects on bacterial ribosomes, protein synthesis inhibitors can also affect mammalian mitochondrial ribosomes, cytosolic ribosomes, or both.* For some antibiotics, such as chloramphenicol, inhibition of mammalian ribosomes represents a major drawback and can cause serious, even lethal side effects. Tetracyclines can also inhibit mammalian ribosomes in vitro, but, fortunately, this class of drugs is concentrated selectively in bacterial cells. For certain other agents, there is usually little or no inhibition of mammalian ribosomes at clinically relevant concentrations. In fact, for these agents, the dose-limiting toxicities seem to be attributable to other mechanisms.

An interesting twist on the issue of selectivity emerged in the 1990s. It was discovered that certain aminoglycoside, macrolide, and lincosamide antibiotics demonstrate some efficacy against eukaryotic microorganisms (e.g., protozoan parasites) that cause opportunistic infections in patients with AIDS and other immunocompromised individuals. There is evidence that the activity of the antibiotics in these cases is

TABLE 33-1 Sites and Mechanisms of Action of Antibacterial Translation Inhibitors

| DRUG OR DRUG CLASS | SITE OF ACTION | MECHANISM OF ACTION |
|---|---|---|
| Drugs targeting the 30S ribosomal subunit | | |
| Aminoglycosides | 16S rRNA | Induce misreading; halt protein synthesis at higher concentrations |
| Spectinomycin | 16S rRNA | Inhibits translocation |
| Tetracyclines | 16S rRNA | Block aminoacyl tRNA binding to A site |
| Drugs targeting the 50S ribosomal subunit | | |
| Macrolides | 23S rRNA | Inhibit translocation |
| Chloramphenicol | 23S rRNA | Inhibits peptidyl transferase by interfering with tRNA positioning |
| Lincosamides | 23S rRNA | Inhibit peptidyl transferase by blocking the growing polypeptide chain, and by inhibiting the A site and P site |
| Streptogramins | 23S rRNA | Inhibit peptidyl transferase; probable overlap with mechanism of action of macrolides |
| Oxazolidinones | 23S rRNA? | Not yet known |

attributable to the sensitivity of organellar protein synthesis in these microorganisms.

The third consideration is that *complete inhibition of protein synthesis is not sufficient to kill a bacterium.* Bacteria have a number of responses to various growth-stifling treatments that allow them to remain dormant until the treatment is removed. One of these responses permits bacteria to survive complete inhibition of protein synthesis. As a result, most inhibitors of protein synthesis are bacteriostatic. Aminoglycosides are the major exception to this rule.

## Antimicrobial Drugs Targeting the 30S Subunit

### *Aminoglycosides*

**Aminoglycosides** are mainly used to treat infections caused by Gram-negative bacteria. These agents are charged molecules that are not orally bioavailable, so they must be administered parenterally. The aminoglycosides include **streptomycin** (the first aminoglycoside, discovered in 1944), neomycin, kanamycin, tobramycin, paramomycin, gentamicin, netilmicin, and amikacin. Of these, **gentamicin, tobramycin,** and **amikacin** are the most widely used agents because of their lower toxicity and broader coverage of target organisms. (Even these agents are not active against anaerobes and many Gram-positive bacteria, however.)

Aminoglycosides bind to the 16S rRNA of the 30S subunit, and elicit concentration-dependent effects on protein synthesis. At low concentrations, aminoglycosides induce ribosomes to misread mRNA during elongation, leading to synthesis of proteins containing incorrect amino acids. It is logical to infer from this effect that aminoglycosides interfere with the mRNA-decoding function of the 30S subunit. Indeed, crystal structures of 30S-aminoglycoside complexes have greatly aided our understanding of the decoding process. At higher concentrations, aminoglycosides completely inhibit protein synthesis, causing ribosomes to become trapped at the AUG start codons of mRNA. Eventually, accumulation of these abnormal initiation complexes—termed "streptomycin monosomes"—halts translation, despite the presence of ribosomes that are not bound to drug.

In contrast to other protein synthesis inhibitors, aminoglycosides are **bactericidal.** This is an important feature in the treatment of serious infections. Although the precise mechanism for bactericidal activity is not known, one appealing model, developed by the late Bernard Davis, has gained some acceptance (Fig. 33-4). The **Davis model** frames the story of cell death in terms of the concentration-dependent effects of aminoglycosides. When drug first enters the cell, it is poorly transported across bacterial membranes. At these initial low concentrations, misreading occurs, leading to synthesis of aberrant proteins. Some of these proteins insert into membranes and cause the formation of membrane pores, which allow aminoglycosides to flood the cell and halt protein synthesis completely. As a result, the damage to the membrane cannot be repaired, and leakage of ions, and later larger molecules, leads to cell death.

Another important aspect of aminoglycoside activity is that these drugs act synergistically with antibiotics, such as beta-lactams, that inhibit cell wall synthesis. Therefore, aminoglycosides and beta-lactams are commonly used in combination (see Chapter 38). The explanation most commonly suggested for this synergy is that inhibition of cell wall synthesis increases the entry of aminoglycosides into the bacteria. This synergy strongly contrasts with the antagonistic action of bacteriostatic inhibitors of protein synthesis, such as the drugs discussed below, when the latter drugs are combined with beta-lactams.

Three general mechanisms have been established for resistance to aminoglycosides. First, and clinically the most common, is the plasmid-encoded production of a transferase enzyme or enzymes that inactivate aminoglycosides by adenylation, acetylation, or phosphorylation. Second, drug entry into the cell can be impaired, perhaps by alteration or elimination of porins or other proteins involved in drug transport. Third, the drug target on the 30S ribosomal subunit can become resistant to drug binding by virtue of mutation or the activity of a plasmid-encoded enzyme.

In addition to the "usual" types of toxicities, such as hypersensitivity reactions and drug-induced fever, the aminoglycosides can cause three specific adverse effects: ototoxicity, nephrotoxicity, and neuromuscular blockade. Of these, **ototoxicity** (manifesting as either auditory or vestibular damage) is the single most important factor restricting aminoglycoside use. There is excellent evidence that this is caused by inhibition of host mitochondrial ribosomes. The aminoglycosides are

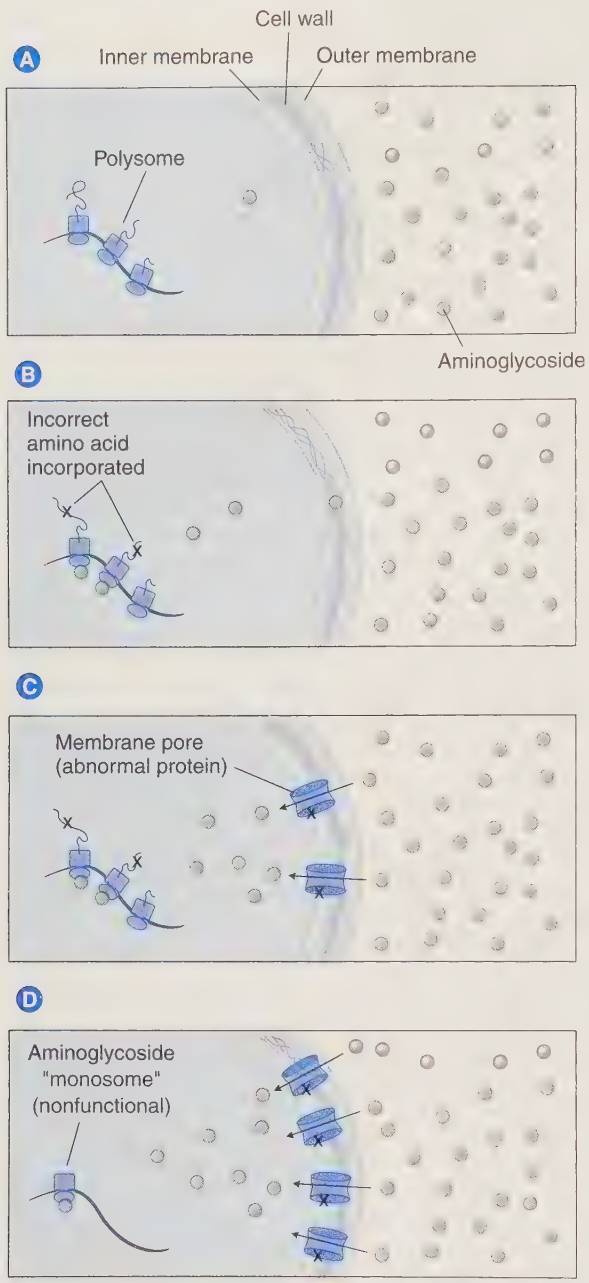


**Figure 33-4. The Davis Model Explains the Bactericidal Activity of Aminoglycosides.** The Davis model of aminoglycoside action proposes that low concentrations of aminoglycosides induce protein misreading and that the misread (abnormal) proteins allow higher concentrations of aminoglycosides to enter the cell and halt protein synthesis. **A.** Initially, aminoglycosides are present at low concentrations inside the bacterial cell, despite therapeutic (high) extracellular concentrations of drug, because the drug molecules have poor uptake across the bacterial membranes. **B.** Low intracellular concentrations of aminoglycoside bind to bacterial ribosomes and cause incorporation of incorrect amino acids (misreading) into nascent polypeptides. **C.** The abnormal proteins insert into the bacterial membranes, forming pores and causing membrane damage. **D.** The damaged membranes allow additional aminoglycoside molecules to flood into the cell, causing complete inhibition of ribosome activity. The effect is irreversible, perhaps because of trapping of drug inside the cell ("caging"). The membrane damage cannot be repaired because new proteins cannot be synthesized, and cell death ensues.

known to accumulate in the perilymph and endolymph of the inner ear and, at high concentrations, to damage highly sensitive hair cells. Aminoglycosides can also cause **acute renal failure,** apparently as a result of drug accumulation in proximal tubular cells. The biochemistry involved in this toxicity is poorly understood, although both mitochondrial poisoning and perturbations of the cell membrane are suspected. At very high concentrations, aminoglycosides can produce a nondepolarizing type of neuromuscular blockade, potentially causing respiratory paralysis. This effect is thought to result from drug competition with calcium at presynaptic sites, leading to a reduction in acetylcholine release, a failure of the postsynaptic end-plate region to depolarize, and muscle paralysis.

### *Spectinomycin*

**Spectinomycin** is a structural relative of the aminoglycosides that also binds to the 16S rRNA of the 30S subunit (albeit at a different location from the aminoglycosides). Spectinomycin permits formation of the 70S complex but inhibits translocation. Unlike the aminoglycosides, however, spectinomycin does not induce codon misreading, nor is it bactericidal. Spectinomycin is administered parenterally and is used clinically only as an alternative regimen for gonorrhea infections.

### *Tetracyclines*

There are seven **tetracyclines** available in the United States: **chlortetracycline**, **oxytetracycline**, **tetracycline**, **demeclocycline**, **methacycline**, **doxycycline**, and **minocycline**. All are close structural relatives and can be considered as a group. Differences in clinical efficacy are minor and relate largely to the pharmacokinetics of absorption, distribution, and excretion of the individual drugs. Tetracyclines are broad-spectrum, bacteriostatic antibiotics that are used widely.

Tetracyclines bind reversibly to the 16S rRNA of the 30S subunit, and inhibit protein synthesis by blocking the binding of aminoacyl tRNA to the A site on the mRNA-ribosome complex. This prevents the addition of further amino acids to the nascent peptide. However, inhibition of protein synthesis does not account entirely for the high selectivity of tetracyclines for bacteria, because these drugs can also halt eukaryotic protein synthesis in vitro at not much higher concentrations. Rather, the high selectivity of tetracyclines derives from the active accumulation of these drugs in bacteria but not in mammalian cells. Tetracyclines enter Gram-negative bacteria by passive diffusion through porin proteins in the outer membrane, followed by active (energy-dependent) transport through the inner cytoplasmic membrane. Uptake into Gram-positive bacteria, such as *Bacillus anthracis* (anthrax), occurs similarly via an energy-dependent system. In contrast, mammalian cells lack the active transport system found in susceptible bacteria.

Given that the bacterial selectivity of tetracyclines results from drug concentrating mechanisms, it follows that resistance can occur through increased drug efflux or decreased drug influx. In fact, plasmid-encoded **efflux pumps** represent the most widespread mechanism employed by tetracycline-resistant microbes. A second form of resistance arises through the production of proteins that interfere with the binding of tetracyclines to the ribosome. Yet a third mechanism is the enzymatic inactivation of tetracyclines.

An important pharmacokinetic feature of the tetracyclines is the interaction of these drugs with foods high in calcium, such as dairy products, and with such medicines as antacids that contain divalent and trivalent cations. Because these products impair the absorption of tetracyclines, these drugs are generally taken on an empty stomach. Once tetracyclines are in the circulation, however, the same interaction with cations—in particular, with calcium—can cause sequestration of the drug in bone and teeth, potentially leading to developmental abnormalities. Teeth can also become discolored because of the UV-absorbing properties of tetracyclines, and these drugs can cause significant photosensitivity.

The use of **doxycycline** minimizes kidney toxicity and gastrointestinal distress, the two most problematic side effects of the tetracyclines. All tetracyclines are excreted in both urine and bile, with the urine being the primary route for most drugs in this class. Compared to other tetracyclines, a lower fraction of doxycycline is eliminated via the urine, making it safer for use in patients with renal failure. Also, doxycycline is excreted in the feces largely in an inactive form, so this agent has the added advantage of minimally altering intestinal flora. Hence, doxycycline has a lower incidence of nausea and vomiting (the most common reason for discontinuing a course of tetracycline), as well as of superinfection with other organisms, especially in immunocompromised patients.

## Antimicrobial Drugs Targeting the 50S Subunit

The best understood of the antibiotics that target the 50S subunit (i.e., the macrolides, chloramphenicol, and lincosamides) bind to a small region of 23S rRNA near the peptidyl transferase active center. Small differences in their binding locations may be responsible for differences in their detailed mechanisms of action, however.

### Macrolides

Macrolides are named for their large lactone rings, to which are attached one or more deoxy sugars. **Erythromycin** is the best-known member of this group. More recent semisynthetic derivatives of erythromycin, **azithromycin** and **clarithromycin,** are broader spectrum and are, therefore, growing in use. Macrolides have proved especially important in the treatment of pulmonary infections, including Legionnaires' Disease. These agents display excellent lung tissue penetration and, as important, they have intracellular activity against *Legionella.*

Macrolides are bacteriostatic antibiotics that block the translocation step of protein synthesis by targeting the 23S rRNA of the 50S subunit. Macrolides bind to a specific segment of 23S rRNA and block the exit tunnel from which nascent peptides emerge.

Macrolide use is complicated by the problem of resistance, which is usually plasmid-encoded. One mechanism employed by resistant strains (e.g., *Enterobacteriaceae*) is the production of esterases that hydrolyze macrolides. Modification of the ribosomal binding site by chromosomal mutation represents a second mechanism of resistance. In addition, bacteria can reduce the permeability of their cell membrane to macrolides, or increase active drug efflux. Finally, methylase production accounts for the vast majority of resistance to macrolides in Gram-positive organisms. Methylase modifies the ribosomal target, leading to decreased drug binding. Constitutive production of methylase also confers resistance to structurally unrelated but mechanistically similar compounds, such as clindamycin and streptogramin B (see below).

Adverse reactions to erythromycin usually involve the gastrointestinal tract or the liver. Gastrointestinal intolerance represents the most frequent reason for discontinuing erythromycin, as the drug can directly stimulate gut motility and cause nausea, vomiting, diarrhea, and sometimes anorexia. Erythromycin can also produce acute cholestatic hepatitis (with fever, jaundice, and impaired liver function), probably as a hypersensitivity reaction. Metabolites of erythromycin can inhibit certain liver cytochrome P450 isozymes and, thus, increase the plasma concentrations of numerous hepatically metabolized drugs. Azithromycin and clarithromycin are generally well tolerated, although these drugs can also lead to liver impairment.

### Chloramphenicol

**Chloramphenicol** is a bacteriostatic broad-spectrum antibiotic that is active against both aerobic and anaerobic Gram-positive and Gram-negative organisms. The most highly susceptible organisms include *Haemophilus influenza, Neisseria meningitidis,* and some strains of *Bacteroides.* However, the potential for serious toxicities has limited the systemic use of chloramphenicol. The drug is still used occasionally in the treatment of typhoid fever, bacterial meningitis, and rickettsial diseases, but only when safer alternatives are not available, as in the case of resistance or allergies.

Chloramphenicol binds to 23S rRNA and inhibits peptide bond formation, evidently by occupying a location that interferes with proper positioning of the aminoacyl moiety of the tRNA in the A site.

Microbes have developed resistance to chloramphenicol by two major mechanisms. Low-level resistance has emerged from large chloramphenicol-susceptible populations by the selection of mutants with decreased permeability to the drug. However, more clinically significant resistance has arisen from the spread of specific plasmid-encoded **acetyltransferases** (at least three types of which have been characterized) that inactivate the drug.

The fundamental mechanism of chloramphenicol's toxicity seems to be inhibition of mitochondrial protein synthesis. One manifestation of this toxicity is the **gray baby syndrome,** which can occur when chloramphenicol is administered at high doses to newborn infants. Because newborns lack an effective glucuronic acid conjugation mechanism for the degradation and detoxification of chloramphenicol, the drug can accumulate to toxic levels and cause vomiting, flaccidity, hypothermia, gray color, respiratory distress, and metabolic acidosis. More frequently, chloramphenicol causes dose-related, reversible depression of erythropoiesis and gastrointestinal distress (nausea, vomiting, and diarrhea). **Aplastic anemia,** a rare but potentially fatal toxicity, occurs via an idiopathic mechanism that is unrelated to dose.

Of special interest are the adverse reactions that chloramphenicol can cause in tandem with other drugs. Like macrolides, chloramphenicol increases the half-lives of certain drugs, such as phenytoin and warfarin, by inhibiting the hepatic microsomal enzymes that metabolize these drugs. Chloramphenicol antagonizes the bactericidal effects of penicillins and aminoglycosides, as do other bacteriostatic inhibitors of microbial protein synthesis.

### Lincosamides

The major lincosamide used clinically is **clindamycin.** Clindamycin blocks peptide bond formation, evidently through interactions with both the A site (like chloramphenicol) and the P site. Clindamycin is also positioned to block the growing peptide chain (like macrolides).

The most important indication for clindamycin is the treatment of severe anaerobic infections caused by *Bacteroides* and by other anaerobes found in mixed infections. Clindamycin has been implicated as a potential cause of **pseudomembranous colitis** caused by *Clostridium difficile* superinfection. An infrequent member of the normal fecal flora, *C. difficile* is selected for during the administration of clindamycin or other broad-spectrum oral antibiotics. *C. difficile* elaborates a cytotoxin that can cause colitis characterized by mucosal ulcerations, severe diarrhea, and fever. This severe side effect is a major concern in the use of clindamycin.

### Streptogramins

In 1999, the FDA approved the first drug from the streptogramin class of protein synthesis inhibitors. This drug was approved for the treatment of patients with serious or life-threatening infections caused by vancomycin-resistant *Enterococcous faecium* or *Streptococcus pyogenes.* The drug is a mixture of two distinct chemicals—in this case, **dalfopristin,** a group A streptogramin, and **quinupristin,** a group B streptogramin. As with the antibiotics described above, the streptogramins bind to the peptidyl transferase center of bacterial 23S rRNA. Mutations and modifications affecting this region can confer resistance. The binding site for the B component overlaps with that of the macrolides and, like the macrolides, the streptogramins seem to block emergence of nascent peptides from the ribosome. The A component can inhibit peptidyl transferase in vitro, but whether the mechanism is the same in vivo remains uncertain.

Streptogramins are unusual among the 50S antibiotics in that they are bactericidal against many, but not all, susceptible bacterial species. A conclusive explanation for this phenomenon remains elusive, but current theory suggests that, unlike the other 50S antibiotics, the streptogramins induce a conformational change in the ribosome that is reversible only after subunit dissociation.

### Oxazolidinones

In 2000, **linezolid** was the first member of the oxazolidinone class of antibacterial agents to be approved for use by the FDA. Linezolid demonstrates excellent activity against drug-resistant Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci (VRE). The mechanism of action of linezolid remains uncertain. The drug appears to act at the 50s ribosomal subunit, however, because mutations in 23s rRNA can confer resistance to linezolid.

## ■ Conclusion and Future Directions

A number of classes of antibiotics target the prokaryotic machinery of transcription and translation, disrupting bacterial gene expression at multiple steps. Most of these drugs demonstrate selective binding to prokaryotic enzymes or RNAs, and have relatively few toxic side effects. All have some toxicities, however, and some (e.g., chloramphenicol) have limited clinical use because of their potential to cause life-threatening toxic effects. Most antibiotics that target transcription and translation are bacteriostatic, whereas a few, such as rifampin and the aminoglycosides, are bactericidal. Drug resistance is a persistent and serious problem for all of these agents. Although the emergence of resistance is an expected consequence of antibiotic use, judicious drug administration, multidrug therapies, and the continued development of new antibacterial agents can retard the emergence of resistance. The development of the new streptogramin and oxazolidinone classes of bacterial ribosome inhibitors represents an important advance in the search for drugs that are effective against resistant bacteria. Further elucidation of the mechanism of action of these drugs will both help to inform the basic cell biology of translation, and to define new biochemical targets for pharmacologic intervention.

## Suggested Readings

Campbell EA, Korzheva N, Mustaev A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 2001;104:901–912. (*Mechanism of rifampicin action.*)

Klein NC. Treatment of Legionnaires' disease. Semin Respir Infect 1998;13:140–146. (*Clinical information relevant for the introductory case in this chapter.*)

Ogle JM, Murphy FV, Tarry MJ, et al. Selection of tRNA by the ribosome requires a transition from an open to a closed form. Cell 2002;111:721–732. (*Structural basis for the mechanism of aminoglycoside-induced codon misreading.*)

Steitz TA, Moore PB. RNA, the first macromolecular catalyst: The ribosome is a ribozyme. Trends Biochem Sci 2003;28:411–418. (*Reviews function of RNA in antibiotic action in the 50s subunit.*)

**Drug Summary Table** **Pharmacology of Transcription and Translation**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **AGENTS THAT INHIBIT THE 30S RIBOSOMAL SUBUNIT**<br>*Mechanism—Bind to 16S rRNA; see Notes for specific action* | | | | |
| **Aminoglycosides**<br>**Neomycin**<br>**Kanamycin**<br>**Tobramycin**<br>**Paramomycin**<br>**Gentamicin**<br>**Netilmicin**<br>**Amikacin** | Gram-negative enteric organisms | Ototoxicity<br>Nephrotoxicity<br>Neuromuscular blockade | Adjust dose in renal insufficiency | Induce misreading of codons; inhibit all translation at higher concentrations<br>Bactericidal (Davis model)<br>Not orally bioavailable |
| **Spectinomycin** | Gonorrhea (second line agent) | | | Inhibits translocation<br>Bacteriostatic |
| **Tetracyclines**<br>**Chlortetracycline**<br>**Oxytetracycline**<br>**Tetracycline**<br>**Demeclocycline**<br>**Methacycline**<br>**Doxycycline**<br>**Minocycline** | *Mycoplasma*<br>*Chlamydia*<br>*Rickettsia*<br>Spirochetes<br>*H. pylori*<br>*Vibrio* | Tooth discoloration<br>Tooth, bone deformities | Not to be taken with dairy products or antacids: chelation of divalent cations, such as $Ca^{2+}$ and $Mg^{2+}$, blocks absorption | Block aminoacyl tRNA binding to A site<br>Bacteriostatic |

(Continued)

**Drug Summary Table** **Pharmacology of Transcription and Translation** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **AGENTS THAT INHIBIT THE 50S RIBOSOMAL SUBUNIT**<br>*Mechanism—Bind to 23S rRNA; see Notes for specific action* | | | | |
| Macrolides<br>**Erythromycin**<br>**Clarithromycin**<br>**Azithromycin** | *Corynebacteria*<br>*Chlamydia*<br>*Streptococcus*<br>*Mycoplasma*<br>*Legionella*<br>*Staphylococcus* | Gastrointestinal intolerance | Inhibit P450 enzymes | Inhibit translocation<br>Bacteriostatic |
| **Chloramphenicol** | *Rickettsia*<br>Eye infections (topical use only) | Gray baby syndrome<br>Aplastic anemia | | Inhibits peptidyl transferase<br>Bacteriostatic<br>Rarely used |
| Lincosamides<br>**Clindamycin** | *Bacteroides*<br>Other anaerobes | Pseudomembranous colitis | Previous pseudomembranous colitis | Inhibit peptidyl transferase<br>Bacteriostatic |
| Streptogramins<br>**Quinupristin**<br>**Dalfopristin** | Multidrug-resistant *Streptococcus, Staphylococcus, Enterococcus* | Hyperbilirubinemia<br>Inflammation at infusion site | | Inhibit translocation<br>Quinupristin and dalfopristin are marketed as a combination therapy |
| Oxazolidinones<br>**Linezolid** | Multidrug-resistant *Streptococcus, Staphylococcus, Enterococcus* | Inhibits monoamine oxidase (MAO)<br>Myelosuppression | Selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) | Precise mechanism unknown |
| **AGENTS THAT INHIBIT TRANSCRIPTION**<br>*Mechanism—Bind to β subunit of bacterial RNA polymerase* | | | | |
| **Rifampin** | Prophylaxis against meningococcal disease<br>Tuberculosis | Rash, fever, nausea, vomiting, jaundice | Increases P450 activity; may decrease plasma levels of other drugs, including oral contraceptive pills | Administered in combination with other anti-tuberculosis drugs |

# 34

# Pharmacology of Viral Replication

Robert W. Yeh and Donald M. Coen

**Introduction**
**Case**
**Physiology of Viral Replication**
  Viral Life Cycle
**Pharmacologic Classes and Agents**
  Inhibition of Viral Attachment and Entry
  Inhibition of Viral Uncoating
  Inhibition of Viral Genome Replication
    *Antiherpesvirus Nucleoside Analogues*
      *Acyclovir*
      *Famciclovir and Penciclovir*
      *Ganciclovir*
      *Cidofovir*
      *Other Antiherpesvirus Nucleoside Analogues*
    *Anti-HIV and HBV Nucleoside Analogues*
      *Zidovudine*
      *Lamivudine*
    *Non-Nucleoside DNA Polymerase Inhibitors*
    *Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)*
  Inhibition of Viral Maturation
  Inhibition of Viral Release
  Antiviral Drugs With Unknown Mechanisms of Action
    *Fomivirsen*
    *Ribavirin*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Viral infections are among the leading causes of morbidity and mortality worldwide. Although progress has been made on antiviral drug development, public health measures and prophylactic vaccines remain the primary means by which society controls the spread of viral infections. The acquired immunodeficiency syndrome (AIDS) epidemic makes this painfully clear. Despite advances in anti-HIV drug therapies, AIDS is an increasingly common cause of death—particularly in some African nations, where as many as one person in five is infected with human immunodeficiency virus (HIV). This enormous prevalence is largely attributable to failures in public health measures and the lack of an effective vaccine against HIV, in a setting where anti-HIV drugs are too expensive.

Despite this bleak statistic, the array of drugs available to combat viruses has been instrumental in saving millions of lives each year and in improving the quality of life for countless others afflicted by treatable viral illnesses. This chapter describes the physiology of viral replication and the steps in the viral life cycle that are targeted by current antiviral medications. Key concepts for the chapter include: (1) viruses replicate intracellularly by utilizing host cell machinery; (2) the intracellular mode of replication reduces the number of potential targets for antiviral drug therapy; and (3) current antiviral drugs exploit differences between the structures and functions of viral and human proteins to achieve selectivity of antiviral action.

## Case

Mr. Moss, a 26-year-old man, complains to Dr. Rose, his primary care physician, of a sore throat, fever, and tiredness for the past several weeks. On physical examination, Dr. Rose notes bilateral cervical lymphadenopathy, consistent with the patient's "flu-like symptoms." Dr. Rose thinks it likely that Mr. Moss has an infection, possibly a simple "cold," the "flu," or strep throat. Because of Mr. Moss' mononucleosis-like symptoms, Dr. Rose also includes cytomegalovirus (CMV), Epstein-Barr virus (EBV), toxoplasmosis, and HIV in her differential diagnosis. Laboratory tests for *Streptococcus*, CMV, EBV, and toxoplasmosis are negative. Mr. Moss is concerned about the possibility of HIV infection, although he denies any unprotected sexual activity, IV drug use, and other potential exposure risks. Dr. Rose tells Mr. Moss that his symptoms will soon resolve with rest, but that he should return for follow-up within 6 months. She explains to Mr. Moss that, if he has recently contracted HIV, his body would not yet have produced sufficient antibodies to show up on an anti-HIV antibody test.

Five years later, Mr. Moss returns to Dr. Rose's office. He has not seen any physician in the interim and now presents with a number of new symptoms. There are multiple open lesions on his lips and in his mouth, and he confides that he has similar lesions in his genital area. An ELISA test is positive for anti-HIV antibodies, and a viral load measurement shows high levels of HIV RNA in his blood. Mr. Moss's CD4 count is 100 per mm$^3$ (normal range, 800–1200 per mm$^3$). Dr. Rose immediately prescribes a drug regimen of zidovudine (AZT), lamivudine (3TC), and ritonavir, explaining to Mr. Moss that a combination of anti-HIV drugs is his best option for keeping down viral load and forestalling more serious disease. In addition, Dr. Rose prescribes oral acyclovir to treat Mr. Moss' oral and genital herpes.

Over the next 3 years, Mr. Moss's HIV viral load falls to undetectable levels and his condition improves. The herpes infections are also kept in check. Today, Mr. Moss appears in good health and, although it takes considerable effort, he takes his medications diligently.

## QUESTIONS

- **1.** What are the mechanisms of action of the three anti-HIV drugs prescribed by Dr. Rose?
- **2.** What is acyclovir, and how does it work?
- **3.** Why does acyclovir not cause significant toxicity in humans, while AZT does?

## PHYSIOLOGY OF VIRAL REPLICATION

Viruses replicate by co-opting the host cell's metabolic machinery. As a result, there are fewer differences between viruses and their human hosts to exploit for drug development than between bacteria and humans. It is also more difficult to develop agents that are active against a broad spectrum of viruses than it is against bacteria. This difficulty arises because viruses are a heterogeneous group of infectious agents, whereas most bacteria share a common cell wall structure and distinct transcriptional and translational machinery.

Despite these obstacles, all viruses encode proteins that are substantially different from their human counterparts. In principle, antiviral drugs could target many of these proteins. In practice, however, only a few viral proteins have thus far served as useful targets for therapy.

Viruses exist as small particles called **virions.** Virions, in turn, consist of a nucleic acid genome packaged into a virus-encoded protein shell called a **capsid.** In some viruses, the capsid is surrounded by an **envelope,** a lipid bilayer membrane that contains virus-encoded envelope proteins. Viral genomes can consist of DNA or RNA and can be single- or double-stranded.

### VIRAL LIFE CYCLE

Almost all viruses have the same general life cycle for replication. Figure 34-1 presents this cycle for the example of a typical DNA-containing virus. Figure 34-2 illustrates the cycle for HIV, which, as a retrovirus, contains RNA that is copied into DNA. (A somewhat different illustration would be drawn for an RNA-containing virus, such as influenza, in which the viral RNA itself is replicated and transcribed.) At the start of infection, the virus attaches to the host cell. This **attachment** is mediated by proteins on the viral surface that bind specifically to a particular host membrane component. For example, the HIV viral envelope contains the glycoprotein gp120, a transmembrane protein that mediates binding and attachment of the virus to host cells expressing CD4 and the chemokine receptors such as CCR5 or CXCR4 (Fig. 34-2). Next, the virion undergoes **entry** by crossing the host cell membrane. In the case of HIV, this process of cell entry depends on gp41, a viral envelope protein that fuses together the membranes of HIV and the target cell.

The virion then loses enough of its capsid proteins—the stage of **uncoating**—that its nucleic acid becomes available for **transcription** into mRNAs, which then undergo **translation** on cellular ribosomes. For retroviruses, uncoating allows reverse transcription to occur. For certain RNA viruses, uncoating is followed directly by translation of the viral RNA.

**Genome replication** is the next step of the cycle. This step requires a supply of ribonucleoside triphosphates for RNA viruses and deoxyribonucleoside triphosphates for DNA viruses. For DNA viruses, the generation of these deoxyribonucleoside triphosphates occurs via two pathways: the salvage pathway, which employs the pharmacologically relevant enzyme thymidine kinase, and the de novo pathway, which includes the enzyme thymidylate kinase. Nucleoside triphosphates are incorporated into new viral genomes by a viral or cellular polymerase (see Chapter 31, Pharmacology of Nucleotide Synthesis, for more detail on nucleotide metabolism). In the case of herpes simplex virus (HSV), the generation of deoxyribonucleoside triphosphates includes phosphorylation of nucleosides via the salvage pathway by a viral thymidine kinase; a viral DNA polymerase then adds deoxyribonucleoside triphosphates to the growing DNA genome. Exploitation of this two-step process has led to the development of the most effective and safe antivirals currently available, because *differences between human and viral kinases and polymerases allow drugs to take advantage of two different steps in a single pathway.*

Viral proteins that are synthesized intracellularly assemble with viral genomes within the host cell in a process known as **assembly.** For a number of viruses, assembly is followed by a process known as viral **maturation,** which is essential for newly formed virions to become infectious. This process usually involves cleavage of viral polyproteins by proteases. For some viruses, maturation occurs intracellularly; for others, such as HIV, it occurs extracellularly. Viruses **egress** from the cell either by cell lysis or by budding through the cell membrane. For influenza viruses, the newly formed viruses require an additional step of **release** from the extracellular surface of the cell membrane.

In summary, nearly all viruses replicate via the following steps: attachment, entry, uncoating, transcription, translation, genome replication, assembly, and egress. Some viruses have additional steps such as maturation and release. The steps of retrovirus infection occur in a different order from those of most other viruses, and retroviruses have additional steps in their life cycle. For example, replication of HIV includes the additional step of **integration,** in which the viral genome is incorporated into the host genome (Fig. 34-2). Specific host and/or viral proteins are involved in each of these steps. Dif-

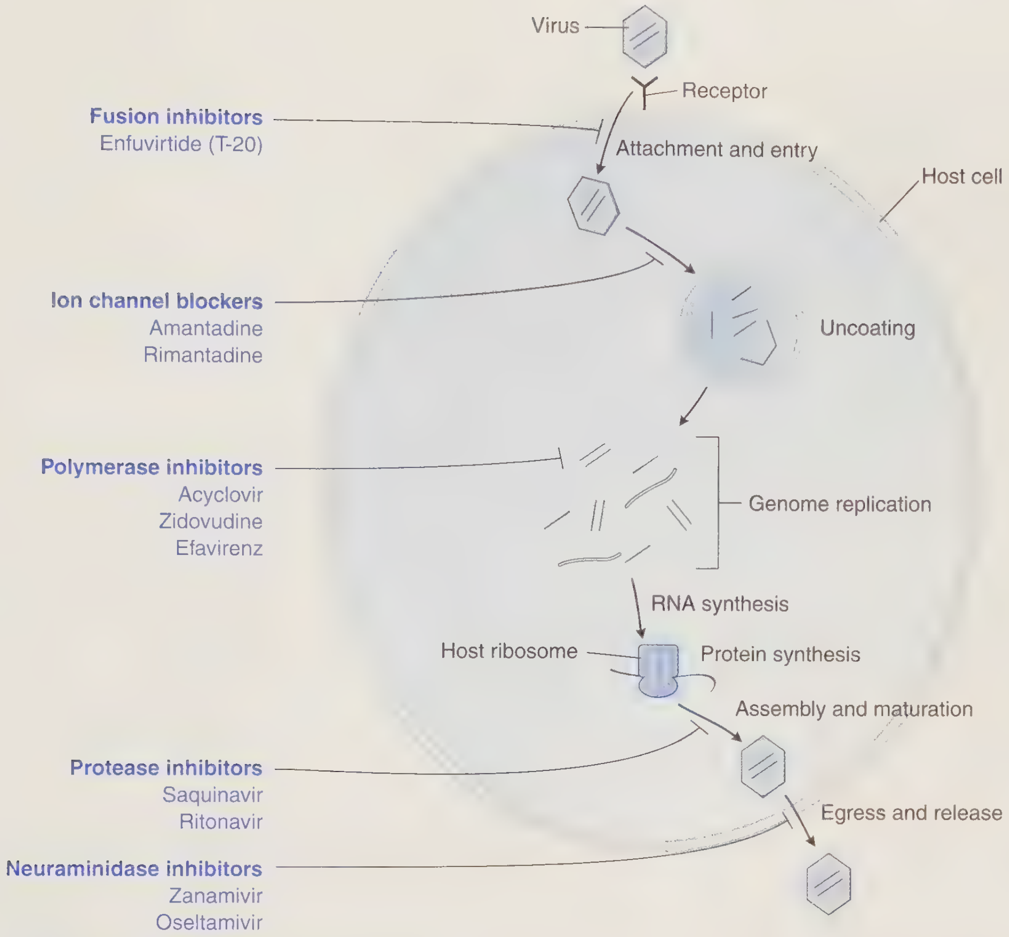

Virus
Receptor
Fusion inhibitors
Enfuvirtide (T-20)
Attachment and entry
Host cell
Ion channel blockers
Amantadine
Rimantadine
Uncoating
Polymerase inhibitors
Acyclovir
Zidovudine
Efavirenz
Genome replication
RNA synthesis
Host ribosome
Protein synthesis
Assembly and maturation
Protease inhibitors
Saquinavir
Ritonavir
Egress and release
Neuraminidase inhibitors
Zanamivir
Oseltamivir

**Figure 34-1. Viral Life Cycle and Pharmacologic Intervention.** The viral life cycle can be divided into a sequence of individual steps, each of which is a potential site for pharmacologic intervention. Shown are drug classes and examples of individual agents that block each step. The majority of the currently approved antiviral agents are nucleoside analogues that target genome replication, typically by inhibiting viral DNA polymerase or reverse transcriptase. A number of other drug classes target other steps in the viral life cycle, including attachment and entry, uncoating, assembly and maturation, and egress and release. It should be noted that the details of viral replication differ for each type of virus, often presenting unique targets for pharmacologic intervention and drug development. For example, the life cycle of HIV (and other retroviruses) includes such additional steps as integration (see Fig. 34-2).

ferences between viral and host proteins at any of these steps can be targeted for antiviral therapy.

Different viruses have vastly different arrays of genes. Some, such as hepatitis B virus (HBV), have compact genomes that encode only coat proteins and a few proteins used in gene expression and replication. Others, such as herpesviruses, encode scores of proteins that perform many different functions. The viral proteins that have thus far served as the best targets for antiviral drugs are enzymes involved in genome replication or maturation, although other steps in the viral life cycle can serve as targets for antiviral agents.

## PHARMACOLOGIC CLASSES AND AGENTS

### INHIBITION OF VIRAL ATTACHMENT AND ENTRY

All viruses must infect cells to replicate. Therefore, inhibiting the initial step of viral attachment and entry provides a conceptual "preventive" measure against infection and could limit the spread of virus throughout the body. Active and passive immunization can inhibit viral infection by providing antibodies against viral envelope proteins; these antibodies can then block the attachment and penetration of virions into cells and increase virion clearance. Alternatively, immunization can be directly virucidal, causing virions to be destroyed or inactivated before the virus can interact with its receptor(s) on target cells.

**Enfuvirtide (T-20),** an anti-HIV peptide, is the first drug that acts by inhibiting viral entry to be approved by the FDA. This agent is structurally similar to a segment of gp41, the HIV protein that mediates membrane fusion. The proposed mechanism for gp41-mediated membrane fusion and T-20 action is illustrated in Figure 34-3. The native gp41 protein in the virion is trapped in a conformation that prevents its ability to fuse membranes or to bind T-20. Binding of HIV to its cellular receptors triggers a conformational change in gp41 that exposes the fusion-active segment (fusion peptide), a heptad repeat region, and a second heptad repeat region mimicked by T-20. The gp41 then refolds, so that the segments mimicked by T-20 bind to the first set of heptad repeats. If the fusion peptide has properly inserted into the host

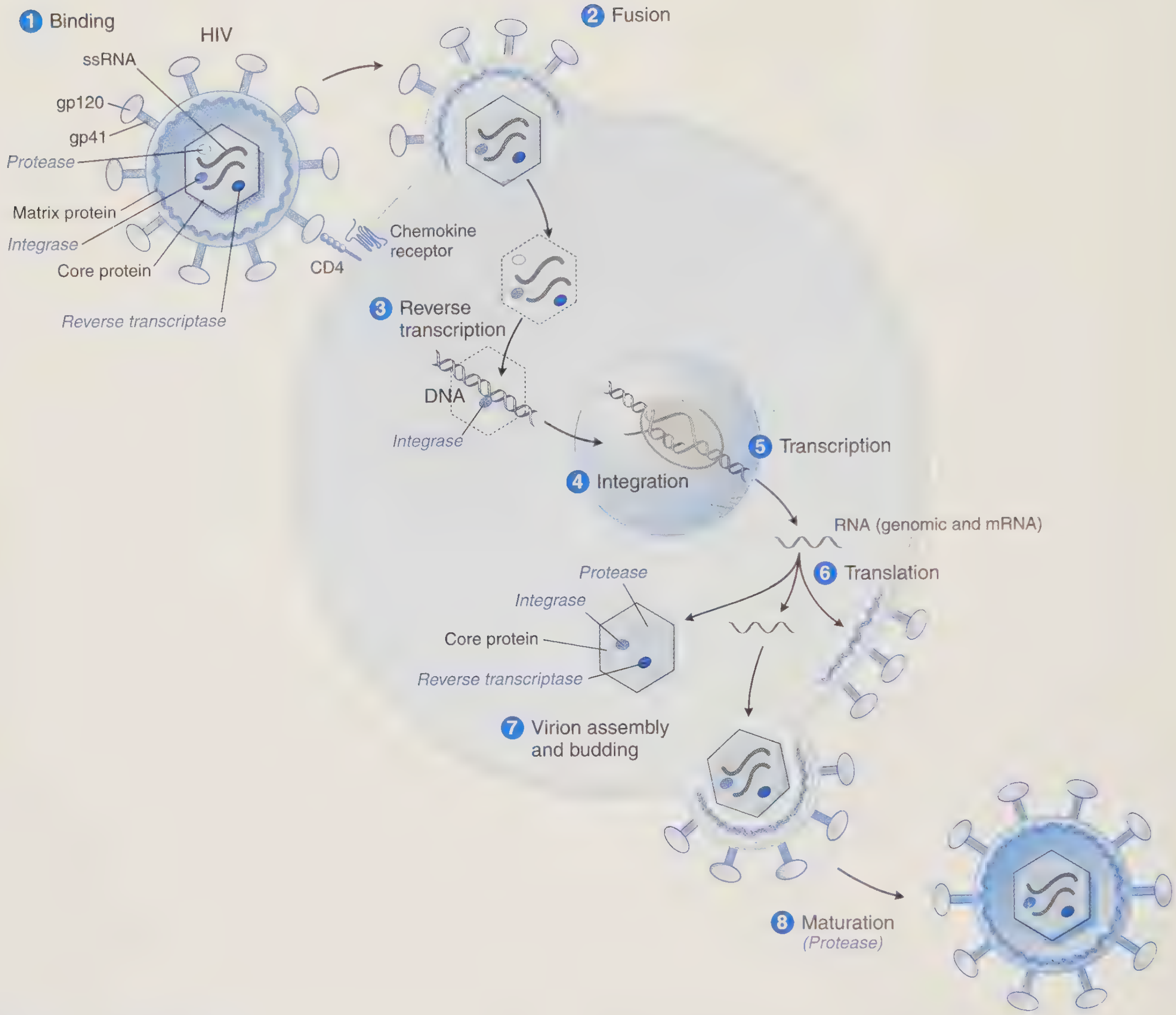

1 Binding
HIV
ssRNA
gp120
gp41
Protease
Matrix protein
Integrase
Core protein
Reverse transcriptase
CD4
Chemokine receptor
2 Fusion
3 Reverse transcription
DNA
Integrase
4 Integration
5 Transcription
RNA (genomic and mRNA)
6 Translation
Protease
Integrase
Core protein
Reverse transcriptase
7 Virion assembly and budding
8 Maturation
(Protease)

**Figure 34-2. Life Cycle of HIV.** HIV is a retrovirus that infects $CD4^+$ cells. **1.** Virus attachment is dependent on binding interactions between viral gp160 (composed of gp41 and gp120 proteins) and host cell CD4 and chemokine receptors. **2.** Fusion of the viral membrane (envelope) with the host cell plasma membrane allows HIV core protein and other proteins to enter the host cell. **3.** Uncoating permits the single-stranded RNA (ssRNA) HIV genome to be copied by reverse transcriptase into double-stranded DNA. **4.** The HIV DNA is integrated into the host cell genome, in a reaction that depends on HIV-encoded integrase. **5.** Gene transcription by host cell enzymes produces genomic HIV RNA and viral mRNA. **6.** The viral mRNA is translated into proteins on host cell ribosomes. **7.** The proteins assemble into immature virions that bud from the host cell membrane. **8.** The virions undergo proteolytic cleavage, maturing into fully infective virions. Currently available anti-HIV drugs target viral fusion, reverse transcription, and maturation. The development of drug resistance can be significantly retarded by using more than one drug that targets a single step (e.g., two or more inhibitors of reverse transcription) or more than one step in the HIV life cycle (e.g., reverse transcriptase inhibitors and protease inhibitors). The diagram shows additional potential targets for future anti-HIV therapy, including proteins involved in HIV binding to $CD4^+$ cells (e.g., gp120, gp41, chemokine receptors) and proteins required for integration of HIV DNA into the host cell genome (e.g., integrase).

cell membrane, this refolding brings the virion envelope and the cell membrane into close proximity, allowing membrane fusion to occur (by mechanisms that remain poorly understood). When T-20 is present, however, the drug binds to the first set of heptad repeats and prevents the refolding process, thereby preventing fusion of the HIV envelope with the host cell membrane.

Because T-20 is a peptide, it must be administered parenterally, typically by twice daily subcutaneous injections. A number of other inhibitors of HIV attachment and entry are under development, including other inhibitors of gp41 and inhibitors of chemokine receptors. Continued clinical trials will be necessary to determine the efficacy and safety of these agents.

## INHIBITION OF VIRAL UNCOATING

**Amantadine** and **rimantadine,** which are active exclusively against influenza A virus (and not against influenza B or C), inhibit uncoating.

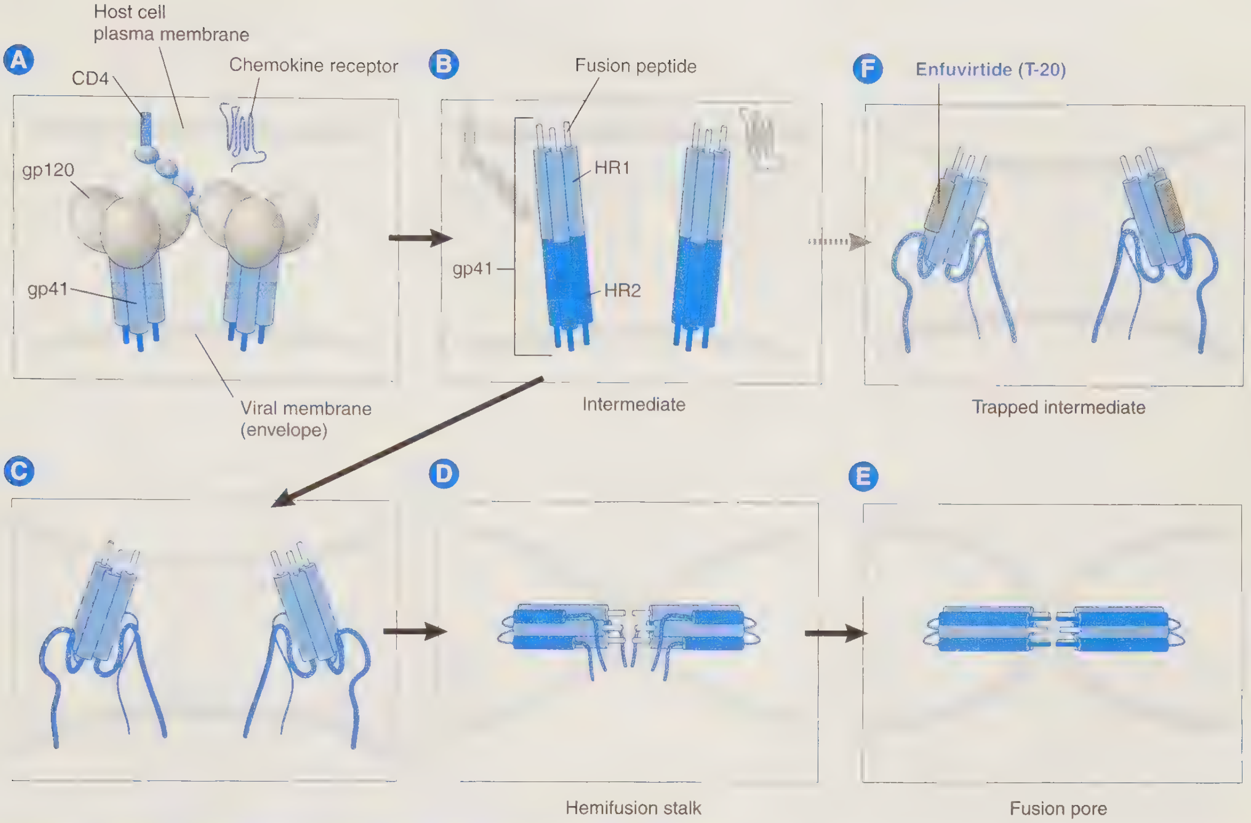


**Figure 34-3. Model for gp41-Mediated Fusion and Enfuvirtide (T-20) Action.** **A.** HIV glycoproteins exist in trimeric form in the viral membrane (envelope). Each gp120 molecule is depicted as a ball attached to gp41. **B.** The binding of gp120 to CD4 and chemokine receptors in the host cell plasma membrane causes a conformational change in gp41 that exposes the fusion peptide, heptad-repeat region 1 (HR1) and heptad-repeat region 2 (HR2). The fusion peptide inserts into the host cell plasma membrane. **C.** The exposed HR regions undergo further conformational changes, characterized mainly by unfolding and refolding of the HR2 repeats. **D.** Completed refolding of the HR regions creates a hemifusion stalk, in which the outer leaflets of the viral and host cell membranes are fused. **E.** Formation of a complete fusion pore allows viral entry into the host cell. **F.** Enfuvirtide (T-20) is a synthetic peptide drug that binds to HR1 and prevents the refolding of HR2 around HR1 (dashed arrow). The drug, therefore, traps the virus–host cell interaction at the binding (attachment) stage, preventing membrane fusion and viral entry.

A well-supported model for amantadine's mechanism of action is diagrammed in Figure 34-4. Influenza enters cells via receptor-mediated endocytosis; this brings virions into endosomes (see Chapter 1, Drug–Receptor Interactions). As endosomes acidify because of the action of an endosomal proton pump, two events occur. First, the conformation of the viral envelope protein **hemagglutinin** drastically changes. This conformational change permits fusion of the influenza virus envelope with the endosome membrane (see the above discussion of HIV-mediated membrane fusion). By itself, this action could liberate viral ribonucleoprotein (including the virion's RNA genome), but that would not be sufficient to permit its transcription. Rather, a second pH-dependent event within the virion is required. This entails the influx of protons through a proton channel called **$M_2$** in the viral envelope, which causes dissociation of the virion **matrix protein** from the rest of the ribonucleoprotein. Amantadine inhibits the influx of protons through $M_2$. Exactly how this inhibition occurs is not clear; amantadine could simply block the channel, although there is evidence for a more complex mechanism.

Amantadine can cause light-headedness and difficulty concentrating. An analogue of amantadine called rimantadine, which has a similar antiviral mechanism, has gained much wider use than amantadine in clinical practice because of its relative lack of side effects, especially neurologic effects that can be problematic in the elderly. Rimantadine is commonly used as a prophylactic agent in settings where there is a large population at risk from influenza morbidity (e.g., nursing homes).

## INHIBITION OF VIRAL GENOME REPLICATION

The vast majority of drugs that inhibit viral genome replication inhibit a polymerase. Every virus uses a polymerase to replicate its genome. A few viruses (e.g., papillomaviruses) use cellular polymerases; for these viruses, drugs targeting these polymerases would also inhibit cellular replication and would be unacceptably toxic. Most viruses, however, encode their own polymerases, making this step an excellent target for antiviral drugs. Viruses whose polymerases have been successfully targeted to yield FDA-approved drugs include certain human herpesviruses, the retrovirus HIV, and the hepadnavirus HBV. Most of these drugs are so-called nucleoside analogues (Fig. 34-5). A few, as discussed below, are non-nucleoside inhibitors of DNA polymerase or reverse

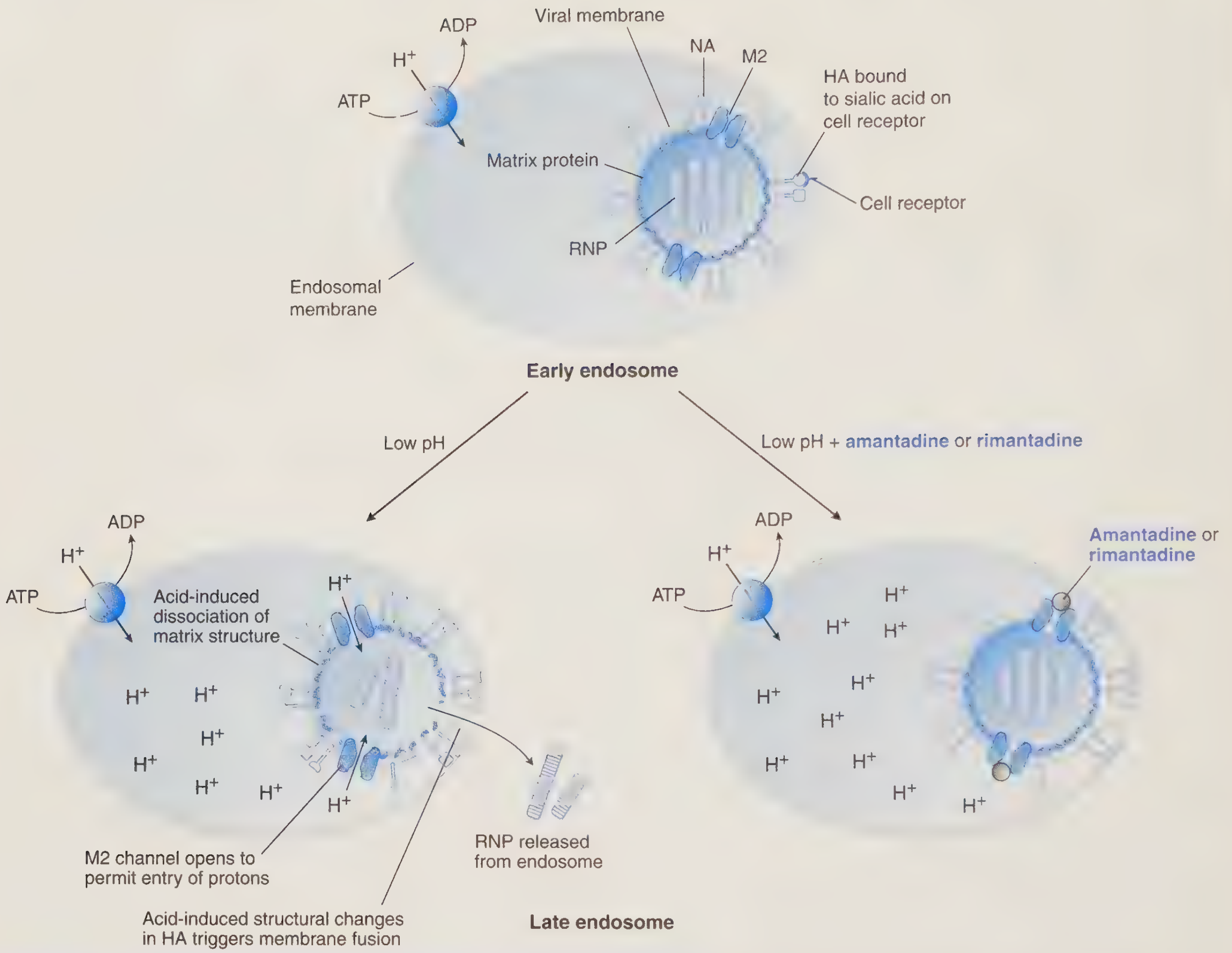
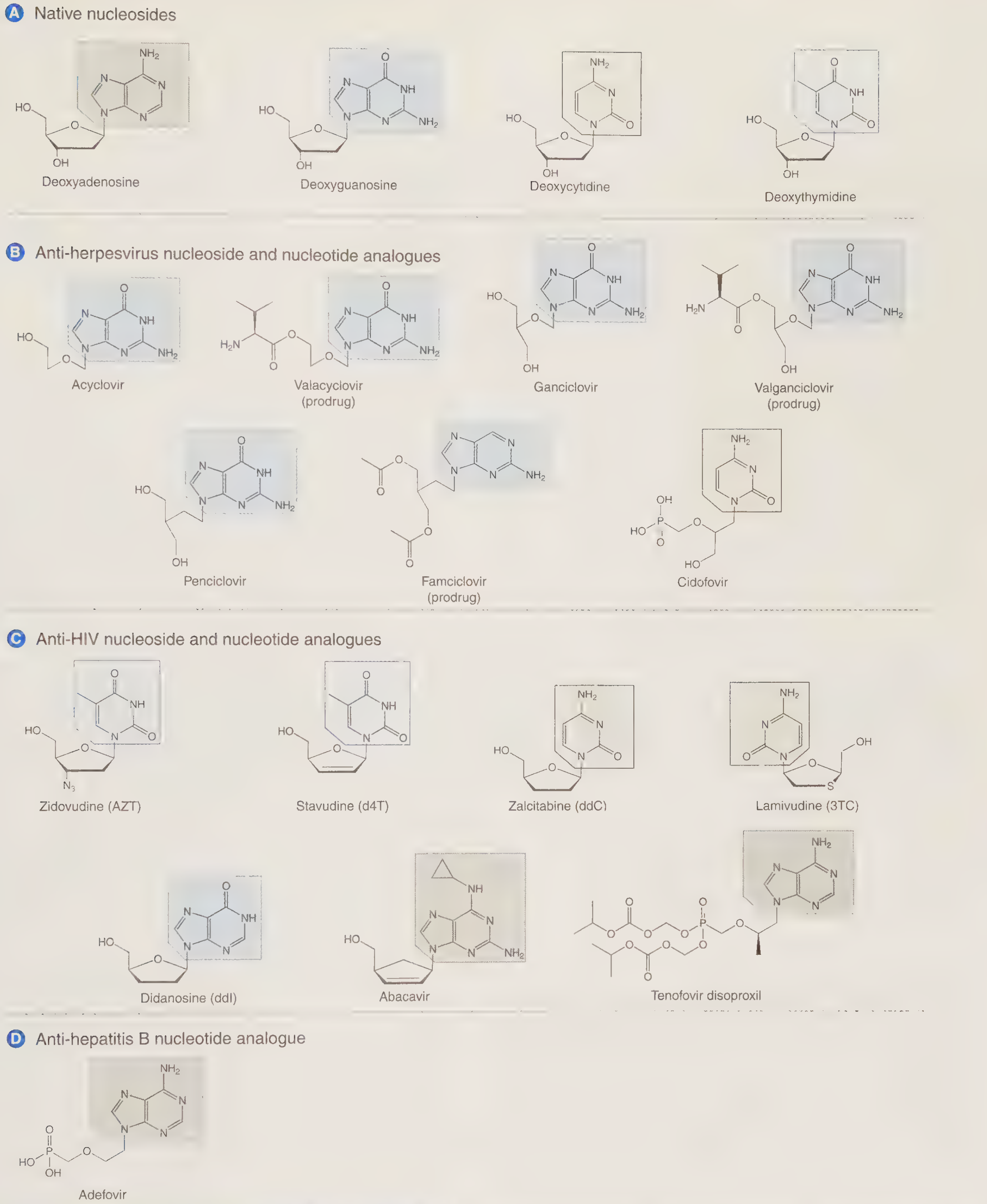

Viral membrane
ADP
H+
ATP
NA
M2
HA bound to sialic acid on cell receptor
Matrix protein
Cell receptor
RNP
Endosomal membrane
Early endosome
Low pH
Low pH + amantadine or rimantadine
ADP
H+
ATP
Acid-induced dissociation of matrix structure
H+
Amantadine or rimantadine
M2 channel opens to permit entry of protons
RNP released from endosome
Acid-induced structural changes in HA triggers membrane fusion
Late endosome

**Figure 34-4. Uncoating of Influenza Virus and Effect of Ion Channel Blockers.** Influenza virus enters host cells by receptor-mediated endocytosis (not shown). The early endosome contains a $H^+$-ATPase that acidifies the endosome by pumping protons from the cytosol into the endosome. A low pH-dependent conformational change in the viral envelope hemagglutinin (HA) protein triggers fusion of the viral membrane with the endosomal membrane. HA binding alone is not sufficient to cause viral uncoating, however. In addition, protons from the low-pH endosome must enter the virus through M2, a proton channel in the viral envelope that opens in response to acidification. The entry of protons through the viral envelope causes dissociation of matrix protein from the influenza virus ribonucleoprotein (RNP), releasing the genetic material of the virus into the host cell cytosol. The viral genome is subsequently transcribed into mRNAs. Amantadine and rimantadine block M2 and, thereby, inhibit viral acidification, dissociation of matrix protein, and uncoating. NA, neuraminidase.

transcriptase. The latter drugs do not structurally resemble physiologic nucleosides, but instead inhibit the activity of DNA polymerase or reverse transcriptase by binding at a site other than the nucleoside-binding site.

All nucleoside analogues must be activated by phosphorylation, usually to the triphosphate form, to exert their effect; phosphorylation allows these agents to mimic deoxyribonucleoside triphosphates, which are the natural substrates of DNA polymerases. *Nucleoside analogues inhibit polymerases by competing with the natural triphosphate substrate; these analogues are usually also incorporated into the growing DNA chain, where they often terminate elongation.* Either or both of these features—enzyme inhibition and incorporation into DNA—can be important for antiviral activity.

The more efficiently cellular enzymes phosphorylate the nucleoside analogue, and the more potent the phosphorylated forms are against cellular enzymes, the more toxic the nucleoside analogue will be. Selectivity, therefore, depends on how much more efficiently viral enzymes phosphorylate the drug than do cellular enzymes, as well as how much more potently and effectively viral DNA synthesis is inhibited than are cellular functions. The challenge in designing nucleoside analogues is to make the drug appear enough like a natural nucleoside that it is activated by cellular enzymes, but not so much like a natural nucleoside that it inhibits cellular processes. All nucleoside analogues employ variations on this theme to achieve their respective degrees of selectivity. The two main categories of nucleoside analogues are the antiherpesvirus agents and the anti-HIV



**A** Native nucleosides

**B** Anti-herpesvirus nucleoside and nucleotide analogues

**C** Anti-HIV nucleoside and nucleotide analogues

**D** Anti-hepatitis B nucleotide analogue

Deoxyadenosine
Deoxyguanosine
Deoxycytidine
Deoxythymidine
Acyclovir
Valacyclovir (prodrug)
Ganciclovir
Valganciclovir (prodrug)
Penciclovir
Famciclovir (prodrug)
Cidofovir
Zidovudine (AZT)
Stavudine (d4T)
Zalcitabine (ddC)
Lamivudine (3TC)
Didanosine (ddI)
Abacavir
Tenofovir disoproxil
Adefovir

**Figure 34-5. Antiviral Nucleoside and Nucleotide Analogues.** **A.** The nucleosides used as precursors for DNA synthesis are depicted here in their *anti* configurations. Each nucleoside consists of a purine (adenine and guanine) or pyrimidine (cytosine and thymidine) base attached to a deoxyribose sugar. These deoxynucleosides are phosphorylated in stepwise fashion to their triphosphate forms (not shown) for use in nucleic acid synthesis. **B.** The antiherpesvirus nucleoside and nucleotide analogues are structural mimics of endogenous nucleosides and nucleotides. For example, acyclovir consists of a guanine base attached to an acyclic sugar. Penciclovir and ganciclovir are also structurally similar to deoxyguanosine. Valacyclovir, famciclovir, and valganciclovir are orally bioavailable prodrugs based on deoxyguanosine. Cidofovir mimics deoxycytidine monophosphate, using a phosphonate (C-P) bond to mimic the physiologic P-O bond of the native nucleotide. These drugs, with the exception of cidofovir, all have some degree of selectivity for viral kinases over human kinases, enabling selective phosphorylation of the drugs in virus-infected cells. Cidofovir does not require activation by viral kinases, because it is a nucleotide analogue. Evidence suggests that the antiviral activity of antiherpesvirus nucleotide analogues is related both to inhibition of viral DNA polymerase and to chain termination upon incorporation of these agents into DNA (not shown). **C.** Anti-HIV nucleoside and nucleotide analogues also mimic endogenous nucleosides. For example, AZT is a deoxythymidine mimic that has a 3′-azido group in place of the native 3′-OH. Anti-HIV nucleoside analogues are activated by cellular kinases; their selectivity exists only at the level of DNA polymerase or reverse transcriptase. Therefore, these agents tend to have more adverse effects than do antiherpesvirus drugs such as acyclovir. **D.** Adefovir is a phosphonate analogue of the endogenous nucleotide deoxyadenosine monophosphate. It is approved for use in the treatment of hepatitis B infection.

agents. Two agents (adefovir and lamivudine) also are used against hepatitis B virus.

## Antiherpesvirus Nucleoside Analogues

Although the diseases that herpesviruses cause are not life-threatening for most people, some, such as genital herpes, caused by HSV, and shingles, caused by varicella zoster virus (VZV), can be painful and emotionally debilitating. For immunocompromised patients like Mr. Moss, however, herpesvirus diseases such as HSV esophagitis and CMV pneumonia or retinitis can cause devastating or even fatal illnesses. Herpesviruses also have the property of **latency.** In latency, viral genomes reside inside a cell and abundantly express at most a few genes, thus avoiding immune surveillance. The viruses can then reactivate long after primary infection and cause disease. No currently available antiviral drug attacks viruses during latency; rather, all available drugs act only on actively replicating virus.

HSV is the best understood herpesvirus in terms of its replication, which corresponds to the schematic in Figure 34-1. Like all herpesviruses, HSV is a large virus that contains double-stranded DNA encoding a variety of proteins involved in DNA replication. These proteins are categorized in two groups. The first group, which includes the viral **DNA polymerase,** participates directly in DNA replication and is absolutely essential for virus replication; the second group, which includes viral **thymidine kinase,** helps catalyze the formation of deoxyribonucleoside triphosphate compounds necessary for DNA replication. Proteins in the second group are not essential for virus replication in cell culture or in certain cells in mammalian hosts, because cellular enzymes can substitute for their activities. Viral DNA polymerase and thymidine kinase differ enough from their cellular counterparts to permit development of selective antiviral nucleoside analogues.

### *Acyclovir*

**Acyclovir (ACV)** is a drug used against HSV and VZV that illustrates the fundamental mechanisms of nucleoside analogues. It is the drug that convinced the medical community that antivirals could be safe and effective. Acyclovir was discovered in a screen of compounds for activity against HSV replication. It exhibits a high therapeutic index (toxic dose/effective dose) because of its high selectivity.

The structure of acyclovir consists of a guanine base attached to a broken and incomplete sugar ring (Fig. 34-5). This acyclic sugar-like molecule accounts for the name of the compound and for aspects of its action.

HSV and VZV each encode a thymidine kinase (TK) that is able to phosphorylate not only thymidine (dT), but also such other pyrimidines as dU and dC, thymidylate (dTMP), and a variety of nucleoside analogues, including some—like acyclovir—that do not contain a pyrimidine base. No mammalian enzyme phosphorylates acyclovir nearly as efficiently as the HSV and VZV thymidine kinases do. However, some phosphorylation does occur in uninfected cells. Regardless, HSV- and VZV-infected cells contain much more phosphorylated acyclovir than do uninfected cells; this finding accounts for much of acyclovir's antiviral selectivity.

Phosphorylation of ACV produces the compound ACV monophosphate. This compound is then converted to ACV diphosphate and ACV triphosphate, probably exclusively by cellular enzymes (Fig. 34-6A). ACV triphosphate then inhibits herpesvirus DNA polymerase. Moreover, it more potently inhibits viral polymerase than cellular DNA polymerase. In vitro, inhibition of HSV DNA polymerase is a three-step process. In the first step, ACV triphosphate competitively inhibits dGTP incorporation (high concentrations of dGTP can reverse inhibition at this early step). Next, ACV triphosphate acts as a substrate and is incorporated into the growing DNA chain opposite a C residue. Finally, the polymerase translocates to the next position on the template, but cannot add a new deoxyribonucleoside triphosphate because there is no 3′-hydroxyl on ACV triphosphate; hence, ACV triphosphate is also a chain terminator. By an unknown mechanism, provided that the next deoxyribonucleoside triphosphate is present, the viral polymerase freezes at this final step in a "dead-end complex," leading to apparent inactivation of the enzyme (Fig. 34-6B). Interestingly, cellular DNA polymerase α does not undergo this inactivation. It is not yet known whether this third, inactivating step is important in vivo, or whether incorporation and chain termination are sufficient to inhibit viral replication. Regardless, studies of ACV-resistance mutations in the viral DNA polymerase gene show that the effects of ACV triphosphate on viral polymerase constitute a major component of acyclovir selectivity.

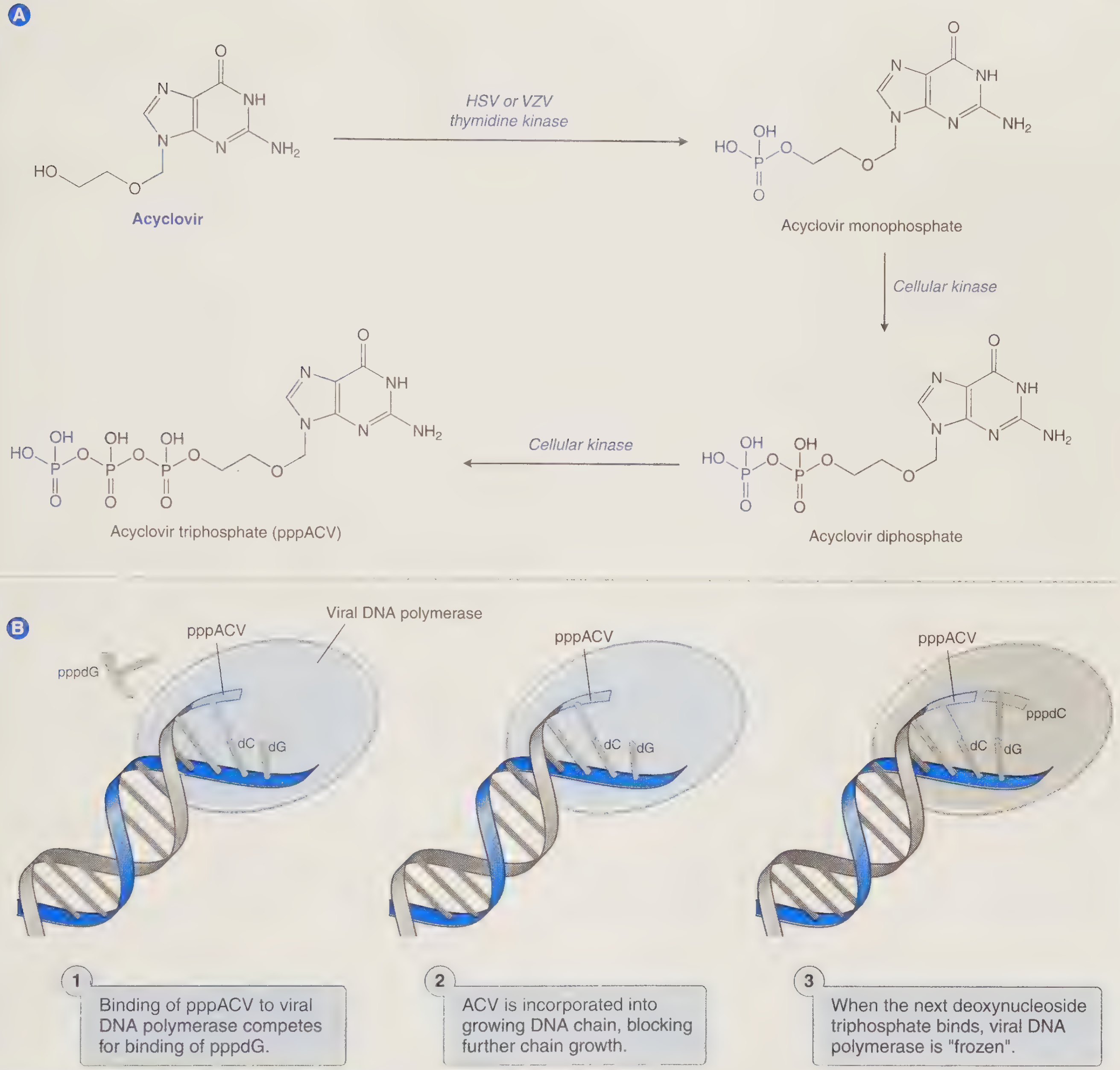
All acyclovir-resistant mutants studied to date contain mutations in either the thymidine kinase (TK) gene, the DNA polymerase gene, or both. Because the TK is not essential for virus replication in cell culture, mutations that completely or partially inactivate the enzyme do not prohibit virus replication. Mutations also exist that alter the enzyme to prevent the phosphorylation of acyclovir while permitting the phosphorylation of thymidine. Because DNA polymerase is essential for virus replication, resistance mutations do not inactivate but instead only alter this enzyme, so that higher concentrations of ACV triphosphate are required for inhibition of the enzyme.

Acyclovir-resistant HSV is a problem clinically, mainly in immunocompromised hosts, but only in a minority of cases (5–10%). Interestingly, in animal models of HSV infection, acyclovir-resistant mutants are frequently attenuated to reduce pathogenicity, but the degree of attenuation depends greatly on the type of mutation. These studies suggest that there are multiple, interesting mechanisms by which the virus can mutate to retain both drug resistance and pathogenicity.

Acyclovir is also produced in its more orally available prodrug form, **valacyclovir** (Fig. 34-5). This compound consists of an acyclovir structure covalently attached to a valine moiety. After oral administration, valacyclovir is rapidly converted to acyclovir. Valacyclovir has approximately five times the oral bioavailability of acyclovir.

### *Famciclovir and Penciclovir*

**Famciclovir** (Fig. 34-5) is the diacetyl 6-deoxy analogue of **penciclovir,** the active form of the drug. Famciclovir is well absorbed orally and modified subsequently by an esterase and an oxidase to yield penciclovir. In humans, this results in ~70% bioavailability. Like acyclovir, the structure of penciclovir consists of a guanine linked to an acyclic sugar-like molecule that lacks a 2′ $CH_2$ moiety.



1 Binding of pppACV to viral DNA polymerase competes for binding of pppdG.

2 ACV is incorporated into growing DNA chain, blocking further chain growth.

3 When the next deoxynucleoside triphosphate binds, viral DNA polymerase is "frozen".

**Figure 34-6. Mechanism of Action of Acyclovir.** **A.** Acyclovir is a nucleoside analogue that is selectively phosphorylated by herpes simplex virus (HSV) or varicella zoster virus (VZV) thymidine kinase to generate acyclovir monophosphate. Cellular (i.e., host) enzymes then sequentially phosphorylate acyclovir monophosphate to its diphosphate and triphosphate (pppACV) forms. **B.** Acyclovir triphosphate acts: (1) as a competitive inhibitor of herpesvirus DNA polymerase; and (2) as a chain terminator once it is incorporated into viral DNA. (Elongation is terminated because acyclovir lacks a 3′-OH group.) Moreover, (3) there is evidence that HSV DNA polymerase is trapped on the acyclovir-terminated DNA chain when the next deoxynucleoside triphosphate binds. pppdC, deoxycytidine triphosphate (dCTP). pppdG, deoxyguanosine triphosphate (dGTP).

Penciclovir's mechanism of action is very similar to that of acyclovir (Fig. 34-6), with only quantitative differences detected by both biochemical assays and analyses of resistant mutants. Penciclovir is more efficiently activated by HSV and VZV TK than is acyclovir, but penciclovir triphosphate is a less selective inhibitor of the viral DNA polymerases than is ACV triphosphate. Famciclovir is used in the treatment of HSV infections and shingles (which are caused by reactivation of VZV), and penciclovir ointment is used for treating cold sores caused by HSV.

### *Ganciclovir*

Human cytomegalovirus (CMV) infections are inapparent in most normal adults, but can cause life-threatening diseases such as pneumonia or sight-threatening retinitis in the immunocompromised. CMV is much less sensitive to acyclovir than are HSV and VZV, primarily because much less phosphorylated acyclovir accumulates in CMV-infected cells than in HSV- or VZV-infected cells. **Ganciclovir,** a nucleoside analogue that was synthesized as a derivative of acyclovir with the intention of developing another anti-HSV drug, is much more potent against CMV than is acyclovir and was the first drug licensed for use against CMV.

Like acyclovir, ganciclovir contains a guanine linked to an acyclic sugar-like molecule that lacks a 2′ moiety. However, it contains the 3′ CHOH group that is missing in acyclovir (Fig. 34-5). Thus, ganciclovir more closely resembles the natural compound, dG, which may account for its greater toxicity (so much toxicity, in fact, that it should be used only for serious infections).

CMV does not encode a homolog of the HSV TK (which phosphorylates ganciclovir very efficiently). However, genetic studies have revealed the existence of a viral protein kinase called UL97 that phosphorylates ganciclovir, leading to a 30-fold increase in the amount of phosphorylated ganciclovir in infected versus uninfected cells. Ganciclovir triphosphate inhibits CMV DNA polymerase more potently than it does cellular DNA polymerases. Thus, as with acyclovir and HSV, *ganciclovir is selective against CMV at two steps: phosphorylation and DNA polymerization.* However, the selectivity at each step is not as great as the selectivity of acyclovir against HSV; accordingly, the drug is more toxic than acyclovir. Toxicity is most commonly manifested as bone marrow suppression, especially neutropenia. As with acyclovir, ganciclovir resistance is a clinical problem in a minority of patients.

Similar to valacyclovir, ganciclovir is also produced as the more orally available agent **valganciclovir,** a valine ester of ganciclovir (Fig. 34-5).

### Cidofovir

Also known as hydroxyphosphonylmethoxypropylcytosine (HPMPC), this phosphonate-containing acyclic cytosine analogue represents a twist on the mechanism of action of antiherpetic nucleoside analogues. Indeed, HPMPC can be considered a nucleotide analogue. With its phosphonate group, **cidofovir** mimics deoxycytidine monophosphate; thus, in effect, it is already phosphorylated (Fig. 34-5). Cidofovir, therefore, does not require viral kinases for its phosphorylation and, accordingly, is active against kinase-deficient viral mutants that are resistant to acyclovir or ganciclovir. Cidofovir enters cells with reasonable efficiency, despite being, in effect, a phosphorylated compound. It is further phosphorylated twice by cellular enzymes to yield an analogue of dCTP, which inhibits herpesvirus DNA polymerases more potently than cellular DNA polymerases. Selectivity has been confirmed by mapping HPMPC-resistance mutations to the CMV polymerase gene.

Cidofovir has been approved for use in the treatment of CMV retinitis in patients with AIDS. Its use requires relatively infrequent dosing (only once per week or less), but, because of its mechanism of renal clearance, it must be administered with probenecid. (Probenecid inhibits a proximal tubule anion transporter and, thereby, decreases cidofovir excretion). Nephrotoxicity is a major problem, so great care must be taken in administering cidofovir.

Two related phosphonate-containing drugs are the acyclic deoxyadenosine monophosphate analogues, **tenofovir** and **adefovir.** Tenofovir was approved as an anti-HIV drug in 2001 and adefovir as an anti-HBV drug in 2002. The mechanisms of action of these drugs against their respective viruses are similar to that of cidofovir against CMV. (See below for discussions of HIV and HBV replication, and of other drugs active against these viruses.)

### Other Antiherpesvirus Nucleoside Analogues

Several other nucleoside analogues with antiherpesvirus activity were developed and approved before the development of acyclovir. These agents have greater toxicity, and so are not widely used, but are listed in the Drug Summary Table at the end of this chapter.

## Anti-HIV and HBV Nucleoside Analogues

HIV is a retrovirus. All retroviruses contain an RNA genome within a capsid surrounded by a lipid envelope studded with glycoproteins. The capsid also contains a small number of enzymes; two that are especially important from a pharmacologic perspective are reverse transcriptase and protease. Both are essential for HIV replication (Fig. 34-2). **Reverse transcriptase** (**RT**) is a DNA polymerase that can copy both DNA and RNA. RT copies the RNA retrovirus genome into double-stranded DNA after the virus enters a new cell. Once the viral DNA is integrated, through the action of the viral enzyme called integrase, cellular RNA polymerase copies it back into RNA to make both full-length genomic RNA and the mRNAs that encode the various viral proteins. The structural proteins assemble onto the full-length genomic RNA and, soon thereafter, the virus buds through the cell membrane and matures into a form capable of infecting new cells. The protease cleaves viral proteins during assembly and maturation (see below). Without these cleavages, the particles that are formed remain functionally immature and noninfectious.

Like herpesviruses, HIV forms latent infections in humans. As with herpesviruses, it seems that no available antiviral drug attacks HIV during latency; available drugs act only on replicating virus.

### Zidovudine

As with the antiherpesvirus drugs described above, **zidovudine** (**azidothymidine, AZT**) is a nucleoside analogue with an altered sugar moiety. Specifically, it is a thymine base attached to a sugar in which the normal 3′ hydroxyl has been converted to an azido group (Fig. 34-5). Thus, as with acyclovir, AZT is an obligatory chain-terminator.

AZT is an excellent substrate for cellular thymidine kinase ($K_m = 3\ \mu M$), which phosphorylates AZT to AZT monophosphate. (HIV does *not* encode its own kinase.) AZT monophosphate is then converted to the diphosphate form by cellular thymidylate kinase and to the triphosphate form by cellular nucleoside diphosphate kinase. Thus, unlike acyclovir and ganciclovir, there is no selectivity at the activation step, and *phosphorylated AZT accumulates in almost all dividing cells in the body, not just infected cells.*

AZT triphosphate targets RT, and is a more potent inhibitor of HIV RT than of the human DNA polymerases that have been tested. The details of the inhibition of reverse transcription are not entirely resolved, but as with acyclovir, incorporation of AZT triphosphate into the growing DNA chain is important.

Thus, AZT can be compared with acyclovir and ganciclovir (Table 34-1). Acyclovir is the most selective of these drugs because it is highly selective at both the activation and inhibition

TABLE 34-1 Selectivity of Action of Antiviral Nucleoside Analogues Is Determined by Specificity of Viral and Cellular Kinases and Polymerases

| DRUG | KINASE SPECIFICITY | POLYMERASE SPECIFICITY |
|---|---|---|
| Acyclovir | Viral TK >> Cellular kinases | Viral DNA polymerase >> Cellular DNA polymerase |
| Ganciclovir | Viral UL97 > Cellular kinases | Viral DNA polymerase > Cellular DNA polymerase |
| Zidovudine (AZT) | Cellular TK | Viral RT > Cellular DNA polymerase |

Drugs are presented in order of selectivity of action. >>, large difference in specificity; >, modest difference in specificity. TK, thymidine kinase. RT, reverse transcriptase.

steps. AZT is probably the least selective because it is nonselective at the activation step and only modestly selective at the inhibition step. Ganciclovir is intermediate in selectivity, with modest selectivity at both the activation and inhibition steps.

Especially because phosphorylated AZT accumulates in almost all dividing cells in the body, its toxicity is a serious clinical issue. In particular, AZT causes bone marrow suppression, which is manifested most commonly as neutropenia and anemia in patients infected with HIV. AZT toxicity seems to be caused not only by the effects of AZT triphosphate on cellular polymerases, but also by the effects of AZT monophosphate, which is not only a substrate for cellular thymidylate kinase but also an inhibitor of this essential enzyme. The limited clinical effectiveness of AZT, and problems with its toxicity and resistance, led to the development of other anti-HIV drugs and to the use of combination chemotherapy for HIV (Box 34-1).

### *Lamivudine*

Several other anti-HIV nucleoside analogues are available, all of which use cellular rather than viral enzymes for activation to their triphosphate forms. These are listed in the Drug Summary Table at the end of this chapter. Of these, **lamivudine,** or **3TC,** appears to exhibit the least toxicity. This may be related to its highly unusual structure: it is an L-stereoisomer, not the standard D-stereoisomer of biological nucleosides (Fig. 34-5). Also unusual is the presence of a sulfur atom. 3TC triphosphate, which is an obligate chain-terminator of DNA synthesis, is a more potent inhibitor of HIV RT than of cellular polymerases. 3TC's lack of certain toxicities may be attributable to the relatively weak inhibition of mitochondrial DNA polymerase by 3TC triphosphate. However, resistance to 3TC develops quickly in patients treated with this drug alone, so it is used in combination with other anti-HIV drugs (Box 34-1).

3TC is also used in patients with chronic hepatitis B virus infections and evidence of active virus replication. HBV is an unusual DNA virus. Within the HBV virion is a partially double-stranded DNA genome and a virion DNA polymerase that also functions as a reverse transcriptase. Upon entry into the cell nucleus, this polymerase completes synthesis of the viral DNA. The resulting DNA does not ordinarily integrate, but it serves rather as an episomal template for transcription by cellular RNA polymerase, which copies it into RNA to make both full-length genomic RNA and the mRNAs that encode the various viral proteins. Structural proteins, including the viral polymerase, then assemble onto the full-length genomic RNA. Within the resulting particles, which are still inside the infected cell, the polymerase copies the RNA into partially double-stranded DNA. Finally, the virus particle buds out of the cell, acquiring a lipid envelope. 3TC triphosphate is a very potent inhibitor of the HBV polymerase.

## Non-Nucleoside DNA Polymerase Inhibitors

Nucleoside analogues can inhibit cellular as well as viral enzymes. As a result, efforts have been made to discover compounds with different structures that can selectively target viral enzymes. The first such compound to be used clinically was **foscarnet** (**phosphonoformic acid, PFA;** Fig. 34-7). Foscarnet inhibits both DNA and RNA polymerases encoded by a wide variety of viruses. It has a relatively broad spectrum of activity in vitro (including against HIV), but clinically is used for certain serious HSV and CMV infections where therapy with acyclovir or ganciclovir has not succeeded (e.g., because of resistance). However, it should also be noted that certain acyclovir-resistant and ganciclovir-resistant polymerase mutants exhibit at least moderate resistance to foscarnet.

Mechanistically, foscarnet differs from nucleoside analogues in that it does not require activation by cellular or viral enzymes: rather, foscarnet inhibits viral DNA polymerase directly by mimicking the pyrophosphate product of DNA polymerization. Selectivity results from the increased sensitivity of viral DNA polymerase relative to cellular enzymes; this biochemical result was confirmed by the existence of foscarnet-resistant DNA polymerase mutants. As might be expected of a compound that so closely mimics a natural compound (pyrophosphate), foscarnet's selectivity is not as high as acyclovir's; it inhibits cell division at doses not much higher than its effective antiherpesvirus dose. Major drawbacks to foscarnet use include its lack of oral availability and its poor solubility; renal impairment is its major dose-limiting toxicity.

## Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Using a rational approach of target-based, high throughput screening, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) **efavirenz, nevirapine,** and **delaviridine** have been developed (Box 34-2 and Fig. 34-7). Like foscarnet, these drugs directly inhibit their target. X-ray crystallographic studies have revealed that NNRTIs bind near the catalytic site

### BOX 34-1. Combination Chemotherapy in the Treatment of HIV

When AZT was first introduced, monotherapy with this drug delayed disease progression in HIV-infected individuals and prolonged the survival of patients with advanced AIDS. In the late 1980s and early 1990s, this was a major advance in treatment. Since then, however, the drawbacks of AZT as monotherapy have become well recognized. AZT causes considerable toxicity— including anemia, nausea, headache, insomnia, arthralgia, and, rarely, lactic acidosis—and it effects only a modest (3- to 10-fold) and transient decrease in the viral load of HIV in plasma. Most patients treated with AZT inexorably progress to AIDS. AZT-resistant virus can be detected in most of these patients, and it is generally accepted that these AZT-resistant variants contribute to the low long-term efficacy of AZT monotherapy.

Similar problems have been encountered with the use of most other anti-HIV drugs as monotherapy. When 3TC, the NNRTIs, or protease inhibitors are used as single agents, although the initial antiviral efficacy is greater than that of AZT (> 30-fold reduction in the amount of HIV in plasma), it is still incomplete, and resistance develops even more quickly than it does with AZT. Toxicity, unfavorable pharmacokinetic properties, and drug–drug interactions are also significant problems with many of the available agents.

Because of these drawbacks, combination chemotherapy (i.e., the use of "drug cocktails"; see Chapter 38, Principles of Combination Chemotherapy) has become the standard of care for HIV-infected individuals. The cocktails are more efficacious than single agents, inducing larger decreases in the viral load of HIV. Combination chemotherapy also decreases the emergence of resistance, both because virus replication is more efficaciously inhibited and, therefore, the chances for mutations to arise during replication are reduced, and because multiple mutations are required to confer resistance to all the drugs in the cocktail. In theory, combination chemotherapy can permit each drug to be used at lower doses, thereby reducing toxicity. It is now widely accepted that patients infected with HIV should start with combination chemotherapy rather than with a single drug. Indeed, several drugs have been approved by the FDA for combination use only. Still under debate is whether patients should be treated with combination chemotherapy as early as possible ("hit early, hit hard")—which also subjects patients to unpleasant side effects and increases the risks of poor compliance (e.g., resistance)—or whether viral loads should be allowed to exceed or CD4$^+$ T cells to drop below certain thresholds before combination chemotherapy is started. Long-term studies, including a significant period of follow-up, may be required to resolve this question.

In antibacterial and antineoplastic combination chemotherapy, it is typical that only agents affecting different targets are combined (see Chapter 38). However, in anti-HIV combination chemotherapy, two or even three anti-RT inhibitors have been combined with evident success. One factor accounting for this success could be the low efficacy of each drug alone; combining these drugs could allow for greater efficacy. (Because some of these drugs have toxicity profiles that differ from one another, it is possible to combine these agents without a significant increase in overall toxicity.) A second factor is that mutations conferring resistance to one drug do not necessarily confer resistance to the other drugs. Thus, AZT-resistant mutants remain sensitive to NNRTIs and even to some other nucleoside analogues. A third possible factor is that mutations conferring resistance to one drug can suppress the effects of mutations conferring resistance to another drug, although the clinical significance of this finding is controversial. A fourth possible factor is that certain resistance mutations decrease the "fitness" of the virus; that is, its ability to replicate in the patient. Thus, maintaining selective pressure in favor of drug-resistant viruses by including the drug may have some benefit.

In many patients undergoing combination anti-HIV therapy (often called highly active antiretroviral therapy or **HAART**), the amount of virus in the blood drops below the limits of detection (fewer than 50 copies of HIV RNA/ml in a standard test). Some scientists have speculated that the virus could be eradicated with drug cocktails if treatment were maintained for a long enough period of time. However, anti-HIV drugs, like antiherpesvirus drugs, attack only replicating virus and not latent virus, and the best evidence is that latent virus can remain in the body for many years. Despite this limitation, and the sometimes prohibitive cost of anti-HIV drugs, combination therapy has been perhaps the best news in AIDS care since the beginning of the epidemic.

of reverse transcriptase (RT). These drugs permit reverse transcriptase to bind a dNTP and primer-template, but inhibit the joining of the two. The NNRTIs are orally available, and their side effects (most commonly, rash) are usually less serious than those of foscarnet or most nucleoside analogues. The main limitation of NNRTIs is that resistance develops rapidly, requiring that they be used in combination with other drugs (Box 34-1). One NNRTI, efavirenz, was the first anti-HIV drug to be taken once a day, an important advantage for HIV-infected individuals who must comply with complex combination chemotherapy regimens.

## INHIBITION OF VIRAL MATURATION

For many viruses, including HIV, the assembly of proteins and nucleic acid into particles is not sufficient to produce an infectious virion. For such viruses, an additional step called **maturation** is required. In most cases, these viruses encode proteases that are essential for maturation. Consequently, there has been a great effort to discover drugs active against these enzymes. Much of the impetus for this effort has resulted from the successes and lessons learned from the development of the anti-HIV protease inhibitors. The approved antiviral drugs that target HIV protease—**saquinavir, ritonavir, amprenavir, indinavir, nelfinavir, lopinavir** and **atazanavir**—are successful examples of rational drug design (Fig. 34-8 and Box 34-3).

HIV protease is an attractive target for pharmacologic intervention for several reasons: First, it is essential for HIV replication. Second, a point mutation is sufficient to inactivate the enzyme, suggesting that a small molecule could successfully inhibit activity. Third, the substrates of HIV protease are conserved and somewhat unusual, suggesting both specificity

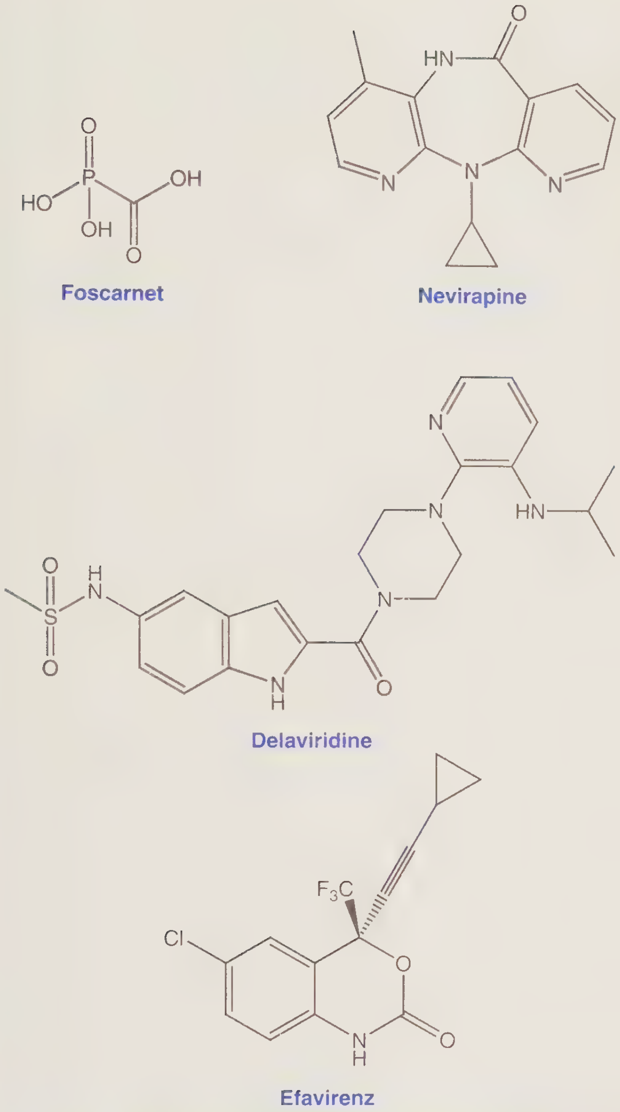


**Figure 34-7. Non-Nucleoside DNA Polymerase Inhibitors (Foscarnet and NNRTIs).** Foscarnet is a pyrophosphate analogue that inhibits viral DNA and RNA polymerases. It is used clinically in the treatment of HSV and CMV infections that are resistant to the antiherpesvirus nucleoside and nucleotide analogues. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz, nevirapine, and delaviridine inhibit HIV-1 reverse transcriptase. The NNRTIs are used in combination with other antiretroviral drugs in the treatment of HIV-1 infection. Note that the structures of the NNRTIs are significantly different from those of the anti-HIV nucleoside and nucleotide analogues (compare with Fig. 34-5).

and a starting point for drug design. Fourth, HIV protease, unlike the human proteases most closely related to it, is a symmetric dimer of two identical subunits, each of which contributes to the active site, again suggesting both specificity and a starting point for drug design. Fifth, the enzyme can be easily overexpressed and assayed, and its crystal structure has been solved. All of these factors increased the likelihood that a drug discovery effort would be successful.

### BOX 34-2. Development of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) were discovered through the method of high-throughput screening. In this method, the gene encoding HIV RT was overexpressed in *E. coli*, and large amounts of RT were purified and used in an RT assay that could be easily automated. Using this assay, many thousands of compounds were screened for their ability to inhibit RT. Candidate compounds were then tested for specificity in a counterscreen by checking their ability to inhibit an unrelated polymerase. The compounds that emerged were then chemically modified to improve their stability, pharmacokinetics, and toxicity. This process eventually yielded NNRTIs that are highly specific, inhibiting HIV-1 RT at low concentrations while not inhibiting the RT of the closely related virus HIV-2.

Ritonavir provides an example of rational drug design. Ritonavir is a peptidomimetic (i.e., it mimics the structure of a peptide; see Box 34-3 and Fig. 34-8). Its design began with the identification of one of the normal substrates of HIV protease, a site for cleaving a longer protein into RT. This site is unusual in that it contains a phenylalanine-proline (Phe-Pro) bond (Fig. 34-8, top); mammalian enzymes rarely, if ever, cleave at such a site. To take advantage of the symmetrical dimer feature of HIV protease structure, correspondingly symmetrical inhibitors were designed in which the Pro was replaced with a Phe. Moreover, CHOH was used instead of the native C=O of the peptide bond in order to mimic the transition state of protease catalysis, which is the catalytic intermediate that binds the enzyme most tightly (Fig. 34-8). However, the designed inhibitors, unlike the original peptide bond and the transition state, cannot be cleaved by the enzyme. How these symmetrical inhibitors evolved into ritonavir is discussed in Box 34-3 (also see Fig. 34-8).

Clever design is no guarantee that a drug will work against the virus by the expected mechanism. However, the protease inhibitors do work as expected. In cell culture, the compounds are potent, although often less potent against virus replication than against the enzyme in vitro. As expected, HIV-infected cells exposed to protease inhibitors continue to make viral proteins, but these proteins are not processed efficiently. Viral particles bud from the infected cells, but these particles are immature and noninfectious. Compelling evidence that protease inhibitors function as expected comes from the observation that mutations conferring drug resistance map to HIV sequences encoding the protease.

Protease inhibitors, used in combination with other anti-HIV drugs (Box 34-1), have had a major impact on AIDS therapy, although they have had unexpected side effects involving fat distribution and metabolic abnormalities.

## INHIBITION OF VIRAL RELEASE

Rational design has also led to the development of inhibitors of influenza virus neuraminidases. The rationale for these inhibitors, which block viral release from the host cell, follows from the mechanism of viral attachment. Influenza virus attaches to cells via interactions between hemagglutinin, a protein on the viral envelope, and sialic acid moieties, which are present on many cell membrane glycoproteins. However, upon egress of influenza virus from cells at the end of a round

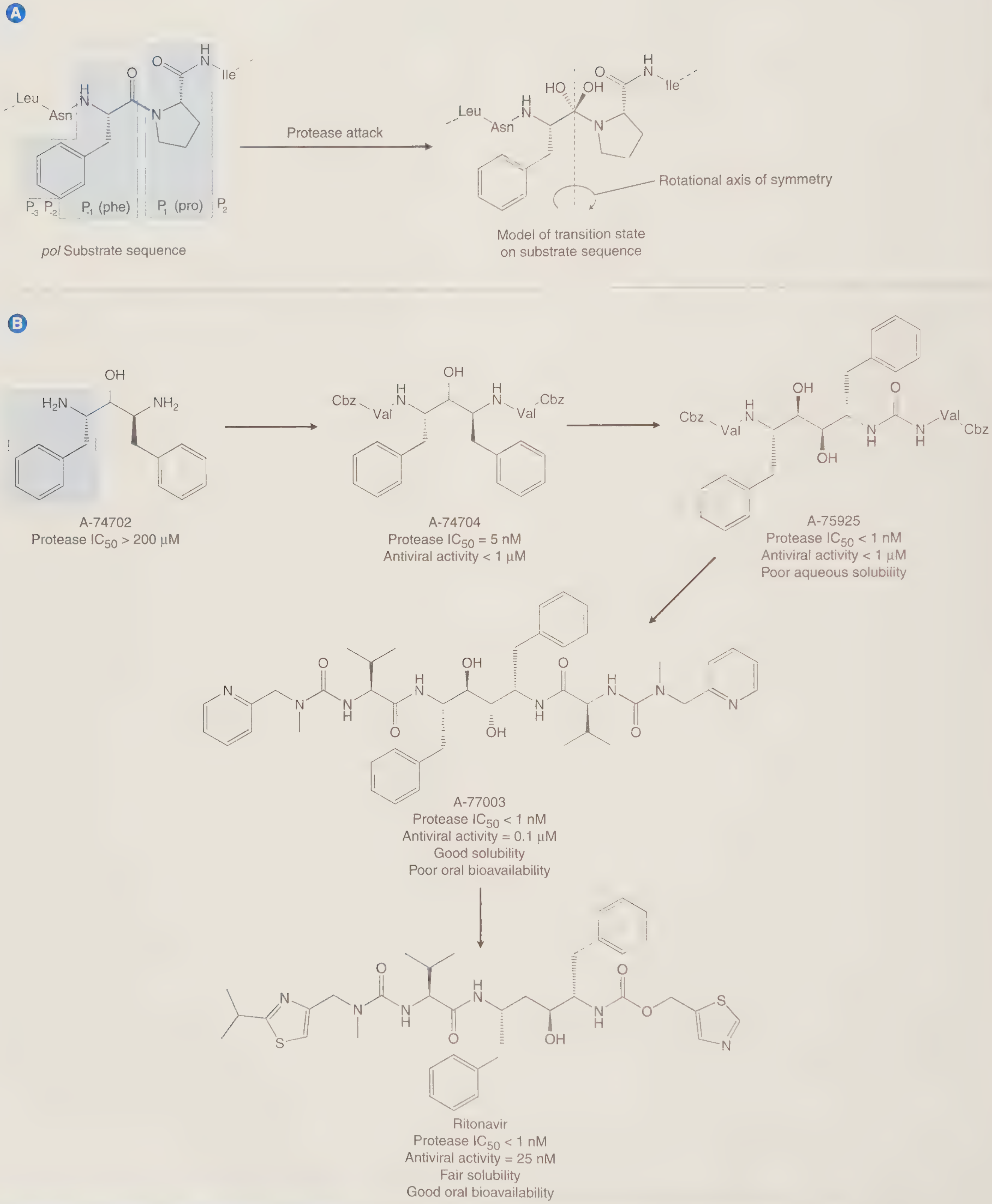

A
Leu
Asn
Ile
P-3 P-2 P1 (phe) P1 (pro) P2
pol Substrate sequence
Protease attack
HO OH
Rotational axis of symmetry
Model of transition state
on substrate sequence
B
H2N
NH2
OH
A-74702
Protease IC50 > 200 μM
Cbz
Val
A-74704
Protease IC50 = 5 nM
Antiviral activity < 1 μM
A-75925
Protease IC50 < 1 nM
Antiviral activity < 1 μM
Poor aqueous solubility
A-77003
Protease IC50 < 1 nM
Antiviral activity = 0.1 μM
Good solubility
Poor oral bioavailability
Ritonavir
Protease IC50 < 1 nM
Antiviral activity = 25 nM
Fair solubility
Good oral bioavailability

**Figure 34-8. Steps in the Evolution of Ritonavir.** **A.** The HIV *pol* gene product has a phenylalanine (phe)-proline (pro) sequence that is unusual as a cleavage site for human proteases. HIV protease cleaves the phe–pro bond. The transition state of this protease reaction includes a rotational axis of symmetry. **B.** Structure-based development of a selective HIV protease inhibitor began with a compound (A-74702) that contained two phenylalanine analogues with a CHOH moiety between them. This compound was then modified to maximize antiprotease activity (measured as $IC_{50}$, the concentration required to cause 50% inhibition of the enzyme), while also maximizing antiviral activity, aqueous solubility, and oral bioavailability. See Box 34-3 for details.

### BOX 34-3. Development of Ritonavir

The development of ritonavir is an example of structure-based ("rational") drug design. Scientists began with a model of the transition state that forms during the cleavage of a substrate by HIV protease (Fig. 34-8). An analogue of the transition state was designed, using just one residue on each side of the cleavage site. Knowing that HIV protease is a symmetric dimer, the scientists chose to use the same residue—phenylalanine—on both sides of the cleavage site, with a CHOH group that mimics the transition state as the center of symmetry. This molecule, A-74702, was a very weak inhibitor of HIV protease, but adding symmetrical groups at both ends to form A-74704 (Fig. 34-8, where Val is valine and CBZ is carbobenzyloxy) resulted in a > 40,000-fold increase in potency ($IC_{50} = 5$ nM). Attempts to modify A-74704 to improve aqueous solubility reduced potency, however, so a related potent inhibitor, A-75925, in which the center of symmetry was a C-C bond between two CHOH groups, became the scaffold for further modifications. Symmetric changes to both ends of the molecule resulted in a soluble, highly potent inhibitor, A-77003. This compound was not orally bioavailable, however. Further modifications, which removed a central OH group and altered other moieties at each end, resulted in a compound—ritonavir—that was less soluble but had improved antiviral activity and good oral bioavailability. Therapeutically achievable plasma concentrations of ritonavir greatly exceed the concentration required for antiviral activity. In the process of structure-based drug design, successive modifications to these molecules took advantage of X-ray structures of HIV protease complexed to each inhibitor. By examining these structures, scientists were able to make informed guesses about what chemical groups to add or subtract. The result was a therapeutically useful HIV protease inhibitor, ritonavir.

of replication, the hemagglutinin on nascent virions again binds to the sialic acid moieties and tethers the virions, preventing release. To overcome this problem, influenza virus encodes an envelope-bound enzyme, called **neuraminidase,** which cleaves sialic acid from the membrane glycoproteins and, thereby, permits release of the virus. Without neuraminidase, the virus remains tethered and cannot spread to other cells. In 1992, the structure of the neuraminidase–sialic acid complex was solved. The structure showed that sialic acid occupies two of three well-formed pockets on the enzyme. Based largely on this structure, a new sialic acid analogue was designed to maximize energetically favorable interactions in all three of the potential binding pockets (Fig. 34-9). This compound, now known as **zanamivir,** inhibits neuraminidase with a $K_i$ of about 0.1 nM. Zanamivir is active against both influenza A and influenza B, with potencies of about 30 nM. Studies of resistant mutants confirm the mechanism of action described above. (Thus far, resistance to neuraminidase inhibitors has not emerged as a major clinical problem.) However, zanamivir has poor oral bioavailability and must be administered by inhaler.

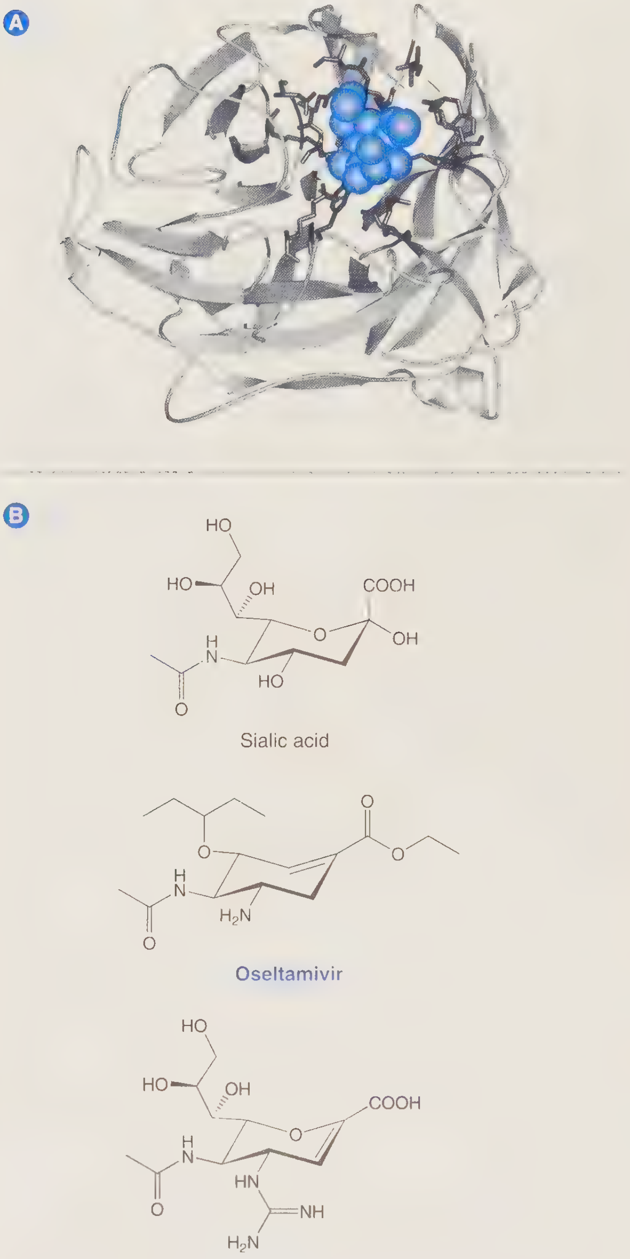


**Figure 34-9. Structure-Based Design of Neuraminidase Inhibitors.** **A.** Shown is a model of sialic acid (space-filling structure) bound to the influenza virus neuraminidase, with the amino acids that bind sialic acid depicted in stick form. Modified versions of the transition state structure bind more tightly to the enzyme, resulting in potent inhibitors of neuraminidase activity. **B.** Structures of sialic acid and the neuraminidase inhibitors oseltamivir and zanamivir.

Efforts to improve on zanamivir's pharmacokinetics resulted in a new drug, **oseltamivir** (Fig. 34-9), whose oral availability is approximately 75%. Oseltamivir, when taken prophylactically, has been shown to reduce the number of flu cases in susceptible populations (e.g., nursing home residents). Both oseltamivir and zanamivir reduce the duration of flu symptoms in patients who are already infected with the virus. However, this reduction is, on average, only 1 day, and requires that the drugs be taken within 2 days of the onset of symptoms. Although it is universally acknowledged that even 1 day less of "the flu" is a benefit, there is considerable disagreement about whether that benefit is worth the cost of these drugs and their potential side effects. Regardless, the neuraminidase inhibitors represent a triumph of rational drug design.

## ANTIVIRAL DRUGS WITH UNKNOWN MECHANISMS OF ACTION

Despite the increasing success of rational drug design, a number of antiviral agents act by unknown or only partially understood mechanisms. Some of these agents, such as fomivirsen, were originally designed to act by a specific mechanism, but may have other pharmacologic effects. Others, such as ribavirin, were discovered empirically.

### Fomivirsen

A new anti-CMV drug, **fomivirsen** was designed to be an antisense oligonucleotide. Antisense oligonucleotides target specific RNAs. Statistically, an oligonucleotide longer than 15 bases that is complementary to a viral RNA will have a binding site that is unique to the virus relative to the entire human genome. This oligonucleotide should be able to base-pair to the virus-specific segment of RNA and disrupt its function, by inhibiting RNA processing or translation or by promoting RNA degradation. If the viral RNA is an mRNA, binding should result in the prevention of synthesis of the protein encoded by the mRNA.

Fomivirsen is the first FDA-approved oligonucleotide drug. It is a phosphorothioate oligonucleotide (i.e., it contains sulfur replacing one of the oxygens in the phosphodiester backbone) designed to bind to an mRNA that encodes **IE2,** a gene regulatory protein of CMV. Despite their large negative charge, oligonucleotides enter cells efficiently. In cell culture, under the right conditions, fomivirsen is more potent than ganciclovir against CMV, with activity at submicromolar concentrations.

Despite its design, it is not at all certain that fomivirsen acts by binding to IE2 mRNA because alterations in its sequence that substantially reduce base pairing do not significantly reduce antiviral activity, whereas alterations that do not substantially reduce base pairing can greatly reduce antiviral activity. A resistant CMV mutant has been isolated, but its mutation is not in the region that is complementary to fomivirsen. Regardless, the drug is licensed for treatment of ophthalmic CMV disease and is used mainly in retinitis. The patient must be highly motivated to receive therapy, however, because the drug is administered intravitreally.

Regardless of its limitations, fomivirsen may pave the way for development of other oligonucleotide drugs. It is worth noting that antisense RNA, other inhibitory RNAs, antiviral ribozymes, or even inhibitory proteins may be deliverable through gene therapy approaches (see Chapter 49, Protein-Based and Gene Therapies). There is considerable activity in this area as well. Both antisense and gene therapy approaches may not only be useful antiviral strategies, but may also be useful approaches to understanding both viral and host cell gene function.

### Ribavirin

Ribavirin has been touted as a "broad spectrum antiviral" and, indeed, it exhibits activity against many viruses in vitro and efficacy against several in vivo. In patients, however, ribavirin has been approved only in aerosol form (in effect, topical application to the lungs) for severe respiratory syncytial virus (RSV) infections, and only in combination with an interferon for chronic hepatitis C virus infections.

Structurally, ribavirin differs from the other nucleoside analogues discussed above in that it has a normal sugar moiety (ribose) attached to a base-like moiety that most resembles purines (adenine or guanine). Its mechanism of action is still not well understood. Ribavirin is converted to a monophosphate by cellular adenosine kinase and is known to inhibit cellular inosine monophosphate dehydrogenase, thereby lowering GTP pools (see Chapter 31). That this mechanism should confer selective antiviral activity might seem unlikely at first, although there is some support for this from studies of viral mutants. It is possible that certain viral enzymes, such as the enzyme that adds 7-methylguanosine caps to mRNA, have higher $K_m$ values (and, thus, lower affinities) for GTP than do most cellular enzymes. Hence, lowering intracellular GTP concentrations below the $K_m$ values of these viral enzymes could have a selective antiviral effect.

A second possible selective mechanism for ribavirin action, at least for viruses related to RSV, could be inhibition of viral RNA polymerase. Interestingly, both ribavirin diphosphate and ribavirin triphosphate have inhibitory activity against this enzyme from certain viruses. However, RSV polymerase as a selective target has not yet been confirmed by analysis of drug-resistant mutants.

A third possible mechanism also involves viral RNA polymerase—in particular, the error-prone nature of this enzyme, which leads to high mutation rates. Ribavirin has been shown to increase the mutation rates of several viruses, including that of hepatitis C when studied in an in vitro replication system. The increased mutation rates are thought to be caused by incorporation of ribavirin into RNA (without chain termination), although ribavirin's effects on GTP pools could also contribute. The proposed mechanism, called "error catastrophe," postulates that the increased mutation rate pushes the already high error rate of the polymerase "over the edge" of an "error threshold," so that few or no functional viral genomes are produced. This concept is interesting, but controversial.

Whether any of the proposed mechanisms of ribavirin action are relevant for the therapeutic effect of the drug on RSV or hepatitis C infections in people is not known. Indeed, for hepatitis C, it is quite possible that the therapeutic effects of ribavirin are mediated by the immune system. Learning more about how ribavirin acts may lead to improved antiviral therapies.

## *Conclusion and Future Directions*

The various steps of the viral life cycle provide the basis for understanding the mechanisms of action of currently available antiviral drugs and for developing new antiviral therapies. The vast majority of antivirals available today inhibit viruses at the genome replication stage, by taking advantage of structural and functional differences between viral and host polymerases. In addition, enfuvirtide (T-20) inhibits viral attachment and entry, amantadine and rimantadine inhibit viral uncoating, protease inhibitors inhibit viral maturation, and neuraminidase inhibitors inhibit viral release. It is important to bear in mind, however, that many of these drugs inhibit only one virus (e.g., HIV), and, in some cases, only one type of that virus (e.g., HIV-1 but not HIV-2). Only a tiny fraction of viruses known to cause human disease can be treated effectively with the antiviral therapies that are currently available. Nevertheless, great strides have been made. As in the case of Mr. Moss, the treatment of HIV with a combination of drugs can reduce viral loads to undetectable levels and delay the progression of AIDS for many years. Although antiviral therapies do not yet represent either a prevention or a cure for this disease, such therapies have already decreased the morbidity and mortality of HIV/AIDS in millions of individuals.

## *Suggested Readings*

Balfour HJ. Antiviral drugs. N Engl J Med 1999;340:1255–1268. (*Discusses non-HIV antiviral agents, with an emphasis on clinical applications and side effects.*)

Challand R, Young RJ. Antiviral Chemotherapy. Biochemical and Medicinal Chemistry Series. Oxford, UK: Oxford University Press, 1998. (*A good book-length treatment of antiviral drugs.*)

Flexner CF. HIV-protease inhibitors. N Engl J Med 1998;338: 1281–1292. (*Detailed discussion of basic protease mechanisms and clinical aspects of protease inhibitors.*)

Hay AJ, Wolstenholme AJ, Skehel JJ, Smith MH. The molecular basis of the specific anti-influenza inhibition of amantadine. EMBO J 1985;4:3021–3024. (*This classic paper illustrates how viral genetics can be used to identify a drug target.*)

LaBranche C, Galasso G, Moore JP, et al. HIV fusion and its inhibition. Antiviral Res 2001;50:95–115. (*Summarizes the current understanding of HIV fusion, and includes a discussion of fusion inhibitors currently under investigation.*)

von Itzstein M, Wu WY, Kok GB, et al. Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 1993;363:418–423. (*Describes the structure-based design of zanamivir.*)

**Drug Summary Table** **Pharmacology of Viral Replication**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF VIRAL ATTACHMENT AND ENTRY**<br>*Mechanism—Inhibit fusion of viral membrane with host cell plasma membrane* | | | | |
| **T-20/Enfuvirtide** | Approved for HIV in combination with other drugs | Injection site reactions | | Peptide; must be mixed and injected twice daily |
| **INHIBITORS OF VIRAL UNCOATING**<br>*Mechanism—Inhibit uncoating of viral genome* | | | | |
| **Amantadine** | Influenza A | Increased seizure activity, hypotension | Anticholinergics, thiazides, triamterene, trimethoprim | Eliminated by renal excretion; must titrate in patients with renal disease<br>Also used in the treatment of Parkinson's disease |
| **Rimantadine** | Influenza A | Increased seizure activity, hypotension | Cimetidine, aspirin, acetaminophen | Metabolized in the liver |
| **INHIBITORS OF VIRAL GENOME REPLICATION—NUCLEOSIDE ANALOGUES**<br>*Mechanism—Inhibit DNA synthesis by inhibiting viral DNA polymerase or reverse transcriptase* | | | | |
| **ANTI-HERPESVIRUS NUCLEOSIDE ANALOGUES** | | | | |
| **Acyclovir**<br>**Valacyclovir** | HSV, VZV | Nausea with oral use, renal failure with intravenous use | Probenecid | Valacyclovir is oral prodrug of acyclovir, with increased bioavailability |
| **Ganciclovir**<br>**Valganciclovir** | CMV | Diarrhea, leukopenia, anemia | AZT, neutropenia, thrombocytopenia | Valganciclovir is oral prodrug of ganciclovir, with increased bioavailability |
| **Penciclovir**<br>**Famciclovir** | HSV, VZV | Fatigue, pruritus, nausea | Probenecid | Famciclovir is oral prodrug of penciclovir |
| **Cidofovir** | CMV retinitis in patients with AIDS | Renal failure, fever, rash, neutropenia | Amphotericin, aminoglycosides | Nucleotide analogue. Available only by intravenous administration. Nephrotoxic drugs should be avoided. |
| **Vidarabine**<br>**Idoxuridine**<br>**Trifluridine** | HSV keratitis, rarely vidarabine for severe HSV or VZV | Local irritation (trifluridine) | Trifluridine hypersensitivity | Early anti-HSV drugs; vidarabine, idoxuridine are now seldom used. Trifluridine used as ophthalmic preparation. |

## ANTI-HIV AND ANTI-HEPATITIS B VIRUS NUCLEOSIDE ANALOGUES

| | | | | |
|---|---|---|---|---|
| **AZT/Zidovudine** | HIV | Neutropenia, anemia, lactic acidosis, nausea, headache | Bone marrow suppressants, interferon, atovaquone | First anti-HIV drug<br>Prolonged use may result in myopathy, severe hepatomegaly with steatosis |
| **3TC/Lamivudine** | HIV, Hepatitis B | Lactic acidosis, hepatotoxicity, pancreatitis (pediatric patients) | ddC (reduced effectiveness), trimethoprim-sulfa | For HIV, used in combination with other anti-HIV drugs |
| **ddI/Didanosine** | HIV | Pancreatitis, lactic acidosis, hepatomegaly with steatosis, optic neuritis, peripheral neuropathy | Drugs that increase risk of pancreatitis. Increase plasma concentration with probenecid. | Decreases stomach acidity, may decrease absorption of drugs that depend on this (ketoconazole, itraconazole) |
| **ddC/Zalcitabine** | HIV | Pancreatitis, lactic acidosis, peripheral neuropathy, hepatomegaly with steatosis | Amphotericin, cimetidine, aminoglycosides | Used in combination with other anti-HIV drugs<br>May cause hepatic failure in patients with underlying hepatitis |
| **Abacavir** | HIV | Fatal hypersensitivity, lactic acidosis, hepatomegaly with steatosis | Ethanol | Fatal hypersensitivity, anaphylaxis in 5% of patients. If hypersensitivity is suspected, abacavir should not be restarted. Used in combination with other anti-HIV drugs. |
| **d4T/Stavudine** | HIV | Peripheral neuropathy, hypersensitivity | Metronidazole, chloramphenicol, isoniazid | Used following prolonged AZT therapy or in combination |
| **FTC/Entricitabine** | HIV | Lactic acidosis, hepatotoxicity, steatosis, skin discoloration | Patients with hepatitis B should be monitored after discontinuation of FTC | Used in combination with other anti-HIV drugs |
| **Tenofovir** | HIV | Lactic acidosis, hepatotoxicity | Renal impairment, ddA, 3TC, efavirenz, lopinavir, ritonavir, indinavir | Nucleotide analogue, supplied as orally available prodrug. Used in combination. |
| **Adefovir** | Hepatitis B | Disease exacerbation after discontinuing therapy; renal toxicity, lactic acidosis | Renal impairment; can select for resistance to certain anti-HIV drugs | Nucleotide analogue, supplied as orally available prodrug. Must monitor patients after discontinuing therapy. |

## INHIBITORS OF VIRAL GENOME REPLICATION—NON-NUCLEOSIDES

*Mechanism—Inhibit viral DNA polymerase or reverse transcriptase*

| | | | | |
|---|---|---|---|---|
| Foscarnet | CMV retinitis and acyclovir-resistant mucocutaneous HSV | Renal impairment, anemia, seizures | Intravenous pentamidine (hypocalcemia), ritonavir, saquinavir | Not orally available. Requires frequent monitoring for renal impairment, with dose adjustments if necessary. |

(Continued)

## Drug Summary Table Pharmacology of Viral Replication *(Continued)*

| Drug | Clinical Use | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs)** | | | | |
| Efavirenz | HIV-1, in combination with other antiretroviral agents | Rash, psychiatric effects (depression, suicidal ideation), dizziness, insomnia | For all of these NNRTIs, CYP3A4-metabolized drugs are contraindicated— verify metabolism of concurrent medications before prescribing NNRTIs | First-line antiretroviral with AZT and 3TC |
| Nevirapine | HIV-1, in combination with other antiretroviral agents | Life-threatening skin reaction, hepatotoxicity | | Must monitor LFTs and signs of liver dysfunction during first 12 weeks of therapy, watch closely for development of rash |
| Delaviridine | HIV-1, in combination with other antiretroviral agents | Renal failure, hepatotoxicity | | Rare erythema multiforme and/or Stevens-Johnson syndrome |
| **INHIBITORS OF VIRAL MATURATION**<br>*Mechanism—Inhibit viral protease* | | | | |
| Saquinavir<br>Ritonavir<br>Amprenavir<br>Indinavir<br>Nelfinavir<br>Lopinavir<br>Atazanavir | All protease inhibitors are used to treat HIV, in combination with other antiretroviral agents | All protease inhibitors can cause dyslipidemia (↑ cholesterol, ↑ triglycerides), redistribution of body fat, hyperglycemia | Many protease inhibitors are inducers/inhibitors of P450 enzymes, with numerous resultant pharmacokinetic drug interactions | Inhibits CYP3A4<br>Inhibits CYP3A<br>Inhibits CYP3A4. Rare life-threatening skin reaction. Available also as prodrug.<br>Inhibits CYP3A4. Nephrolithiasis, especially in pediatric patients.<br>Inhibits CYP3A<br>Administered in combination with ritonavir; ritonavir inhibits CYP3A, thus increasing plasma levels of lopinavir<br>Once-daily administration; can cause hyperbilirubinemia |
| **INHIBITORS OF VIRAL RELEASE**<br>*Mechanism—Decrease viral release by inhibiting neuraminidase* | | | | |
| Oseltamivir | Influenza A and B | Nausea, vomiting, headache | | Oseltamivir is approved for both prophylaxis and treatment; zanamivir is indicated only for treatment |
| Zanamivir | Influenza A and B | Nausea, vomiting, headache | Asthma, COPD | |
| **ANTIVIRAL AGENTS WITH UNKNOWN MECHANISMS OF ACTION**<br>*Mechanism—Unknown or partially understood* | | | | |
| Fomivirsen | CMV retinitis (second-line) | Ocular inflammation | Hypersensitivity to components | Administered intravitreally |
| Ribavirin | RSV, Hepatitis C (in combination with interferons) | Fatigue, headache, insomnia, hemolytic anemia (when used in combination with interferon alfa-2b) | Hemoglobinopathy | Administered by aerosol for RSV in children; requires careful monitoring of respiratory function. Aerosolized form is not approved for use in adults. |

# 35

# Pharmacology of the Bacterial Cell Wall

Ramy A. Arnaout and Robert R. Rando

**Introduction**
**Case**
**Biochemistry of Bacterial Cell Wall Synthesis**
- Cell Wall Structure and Function
- Cell Wall Biosynthesis
  - *Synthesis of Murein Monomers*
  - *Polymerization*
  - *Crosslinking*
- Mycobacterial Cell Wall
- Autolysins and Cell Wall Degradation

**Pharmacologic Classes and Agents**
- Inhibitors of Murein Monomer Synthesis
  - *Fosfomycin and Fosmidomycin*
  - *Cycloserine*
  - *Bacitracin*
- Inhibitors of Murein Polymer Synthesis
  - *Vancomycin and Teicoplanin*
- Inhibitors of Polymer Crosslinking
  - *Beta-Lactam Antibiotics: General Considerations*
  - *Beta-Lactam Antibiotics: Specific Agents*
    - *Penicillins*
    - *Cephalosporins*
    - *Monobactams and Carbapenems*
- Antimycobacterial Agents: Ethambutol, Pyrazinamide, and Isoniazid

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

In 1928, Alexander Fleming made a chance discovery that would revolutionize the treatment of bacterial infections. That discovery was **penicillin,** the first of a long line of antibiotics that act by inhibiting synthesis of the bacterial **cell wall.** The cell wall's unique chemical and structural properties make it an attractive and prominent target for antibacterial chemotherapy. The emergence and spread of antibiotic resistance increasingly complicates the clinical use of cell wall synthesis inhibitors, however. This chapter reviews the biochemistry of bacterial cell wall synthesis and describes the mechanisms of action, uses, and limitations (resistance mechanisms) of the antibiotics that interfere with this process.

## Case

It is April 1953. The Korean War has reached an uneasy stalemate. In the general hospital in Tokyo, Dr. Alan Pierce's ward has just received a new casualty from the front. Three days earlier, 22-year-old Private Morgan Harris had been caught by sniper fire while on reconnaissance and had been hit just above the left knee. At the MASH unit, the wound had been debrided and dressed, and Pvt. Harris had been started immediately on a course of high-dose penicillin. Nevertheless, by the time he reaches Tokyo, Pvt. Harris has grown faint and delirious and has developed a fever to 103°F. On initial inspection, Dr. Pierce notices a sickly sweet odor about Pvt. Harris' leg. When he removes the dressing, he finds the leg bloated below the knee and the wound soaked in putrid, bloody pus. His diagnosis is gangrene, an infection caused by the Gram-positive bacterium *Clostridium perfringens.* Dr. Pierce orders immediate amputation in hopes of saving the young private's life.

Dr. Pierce is troubled by the case. In the past year, he has seen any number of wounds worse than that of Pvt. Harris, but they have always responded well to aggressive treatment with penicillin. As he is thinking this over, he receives word of more incoming patients—eight men, rumored to be suffering from tuberculosis, who have just been released as part of the Operation Little Switch prisoner exchange. Dr. Pierce knows he has streptomycin on hand, but decides to see what he can do about getting a 6-month supply of the new antituberculosis drug, isoniazid, from the States.

## QUESTIONS

1. What is penicillin, and what is its mechanism of action?
2. Why did penicillin fail for Pvt. Harris when this drug had worked for others before him?
3. Why did Dr. Pierce request a supply of isoniazid from the States?

## BIOCHEMISTRY OF BACTERIAL CELL WALL SYNTHESIS

### CELL WALL STRUCTURE AND FUNCTION

The bacterial cell wall is a three-dimensional meshwork of peptide-crosslinked sugar polymers that surrounds the cell just outside its cytoplasmic membrane (Fig. 35-1). Chemically, the cell wall is known interchangeably as **peptidoglycan,** after its peptide and sugar composition, or **murein,** after the Latin *murus,* meaning "wall." The cell wall is a feature of nearly all clinically important bacteria. The major exceptions are *Mycoplasma pneumoniae,* which can cause atypical pneumonia, and the intracellular form (or "reticulate body") of *Chlamydia trachomatis,* which can cause a sexually transmitted disease. The cell wall is critically important for bacteria because of its tensile strength. This strength allows the cell to maintain its intracellular **osmotic pressure** in environments of variable tonicity. Chemically, the tensile strength of the bacterial cell wall resides in its peptide crosslinks; this fact makes inhibition of peptide crosslinking an attractive pharmacologic strategy. In fact, the largest and most widely used class of bacterial cell wall synthesis inhibitors, the **beta-lactam antibiotics,** act by inhibiting **transpeptidase** enzymes that mediate peptide crosslinking.

Bacteria are conventionally divided into two groups, **Gram-positive** and **Gram-negative,** based on the relative abilities of these bacteria to retain the purple **Gram stain** after being washed with an organic solvent such as acetone. Gram-positive bacteria retain the stain and appear purple, whereas Gram-negative bacteria lose the stain and appear pink. Gram staining is frequently used to help identify the bacteria present in a specimen of body fluid such as urine, sputum, or pus. This is one way Dr. Pierce confirmed the diagnosis of *C. perfringens* back in 1953, and the technique is still standard practice today. The ability to retain Gram stain results from two distinguishing characteristics of cell wall architecture (Fig. 35-1). First, the cell wall of Gram-positive bacteria is simply a coat of murein, whereas Gram-negative bacteria possess a second lipid bilayer, called the outer membrane, outside the murein coat. The second difference is that the murein layer of Gram-positive bacteria is generally much thicker than that of Gram-negative bacteria.

Because it is composed of lipid, the outer membrane of Gram-negative bacteria hinders passage of such hydrophilic substances as nutrients and waste products. (The murein layer, by contrast, is porous enough to allow diffusion of many hydrophilic molecules.) To enhance uptake of hydrophilic nutrients and excretion of hydrophilic waste products, Gram-negative bacteria have **pores,** composed of proteins called **porins,** that traverse the outer membrane (see Fig. 35-1). Pores are important pharmacologically because it is through these pores that most hydrophilic antibiotics gain access to the murein layer and the structures beneath. Also important pharmacologically are the **lipopolysaccharides** in the outer leaflet of the outer membrane of Gram-negative bacteria; these amphipathic molecules protect the bacteria from disruption by such hydrophilic host molecules as bile salts and are important for bacterial adherence to host cells and evasion of the host immune response. Hence, the relative hydrophilicity and hydrophobicity of the various classes of antibacterial drugs

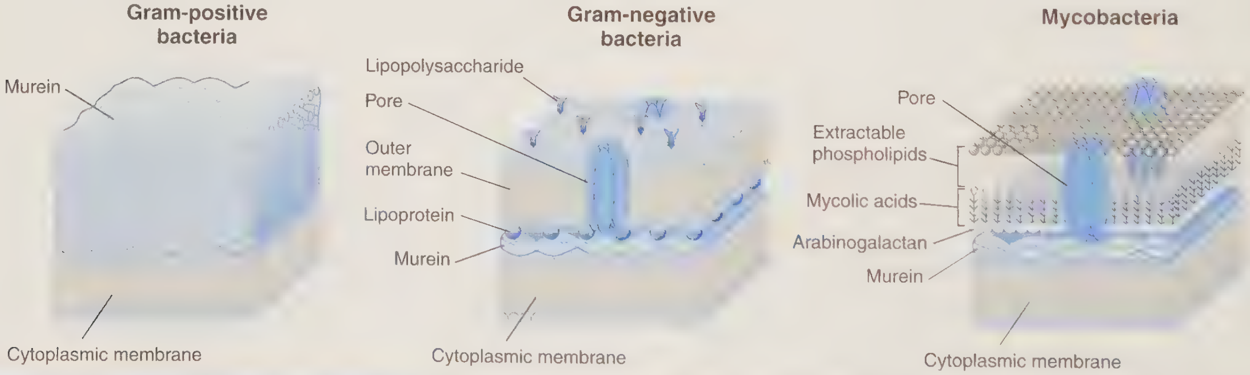


**Figure 35-1. Bacterial Cell Wall Architecture.** In Gram-positive bacteria (left), the cell wall is composed of a thick layer of murein, through which nutrients, waste products, and antibiotics can diffuse. Lipoteichoic acids in the outer leaflet of the cytoplasmic membrane intercalate through the cell wall to the outer surface of Gram-positive bacteria (not shown); the hydrophilic side chains of these molecules are involved in bacterial adherence, feeding, and evasion of the host immune system. In Gram-negative bacteria (center), the murein layer is thinner, and is surrounded by a second, outer lipid bilayer membrane. Hydrophilic molecules cross this outer membrane through channels, which are formed by a cylindrical arrangement of pore proteins (porins). Gram-negative bacteria also have lipopolysaccharide (LPS) in the outer membrane; LPS is a major antigen for the immune response to Gram-negative organisms. The cell wall of mycobacteria (right), which include the causative agents of tuberculosis (*M. tuberculosis*) and leprosy (*M. leprae*), is analogous to that of Gram-negative bacteria. The main difference between the surface architecture of mycobacteria and that of Gram-negative bacteria is that, in mycobacteria, the two leaflets of the outer membrane are asymmetric in size and composition; the inner leaflet of the outer membrane is composed of arabinogalactan and mycolic acids, whereas the outer leaflet is composed of extractable phospholipids.

help to determine the cell wall architecture against which these antibiotics are most effective, as discussed below.

## CELL WALL BIOSYNTHESIS

Cell wall biosynthesis takes place in three major phases. First is the synthesis of murein monomers from amino acids and sugar building blocks; second is the polymerization of the murein monomers into linear peptidoglycan polymers; and third is the crosslinking of the polymers into two-dimensional lattices and three-dimensional mats (Fig. 35-2). The first phase is intracellular and takes place in the cytoplasm; the second is lipid-mediated and takes place at the cytoplasmic membrane; and the third is extracellular and takes place in the **periplasmic space** between the cytoplasmic membrane and the murein layer. (If a bacterium had no cell wall, no periplasmic space would exist. In practice, most bacterial cell wall synthesis consists of remodeling. That is, bacteria add new cell wall components to a previously existing cell wall.) In principle, any of the biochemical steps in any of the three phases of bacterial cell wall synthesis could be a target for in hibition by an antibacterial drug; however, only a few of the biochemical steps are blocked by existing drugs (see below). The details of bacterial cell wall synthesis can be daunting; therefore, it is important to keep these three phases—*monomer synthesis, monomer polymerization, and polymer crosslinking*—in mind during the discussion that follows.

### Synthesis of Murein Monomers

Murein (peptidoglycan) is synthesized from amino acids and sugars. Murein monomer synthesis begins with the modification of glucose to two derivatives, ***N*-acetylglucosamine** (*N*-acetyl-β-D-glucosamine, **NAG**) and ***N*-acetylmuramic acid** (**NAM,** see Fig. 35-2). NAG is synthesized from the closed-chain glucose β-D-glucopyranose by amidation and phosphorylation to glucosamine-1-phosphate, which is then acetylated and activated by the addition of **uridine diphosphate** (**UDP**) to form UDP–NAG. NAM is itself a NAG derivative and is formed from UDP–NAG in two reactions. First, **phosphoenolpyruvate** is added to UDP–NAG to form UDP–NAG pyruvate enol ether, a step catalyzed by the enzyme **MurA** (also known as **enolpyruvate transferase;** Box 35-1); second, **MurB** (also known as **UDP-NAG-enolpyruvate reductase**) reduces this molecule to UDP–NAM. This completes the synthesis of the sugar components.

Next, the peptide component is added. This is performed by a series of peptide transferases (**MurC, MurD,** and **MurE**), which sequentially add the amino acids L-alanine, D-glutamate, and a diamino acid—either L-lysine or **diaminopimelic acid (DAP)**—to UDP–NAM. DAP differs from lysine only in having an additional carboxyl group. Most Gram-positive bacteria use L-lysine, whereas a minority of Gram-positive and all known Gram-negative bacteria use DAP. This is noteworthy because DAP is not found in humans and, therefore, offers a unique target for future drug development.

Peptide formation continues with the addition of a D-alanyl-D-alanine dipeptide (D-Ala-D-Ala) to the diamino acid. The dipeptide is synthesized from two molecules of L-alanine in two reactions. Because amino acids are usually available in the environment in the L-conformation—which is the conformation found in most mammalian proteins—the first reaction requires that two molecules of L-alanine are transformed into D-alanine. This reaction is catalyzed by the enzyme **alanine racemase.** In the second reaction, an enzyme called D-Ala-D-Ala synthetase (or **D-Ala-D-Ala ligase**) joins the two D-alanines together; this reaction requires ATP. The resulting D-Ala-D-Ala dipeptide is added by the enzyme **MurF** to the peptide-substituted UDP-NAM to form UDP-NAM-L-Ala-D-Glu-L-Lys-(or DAP-)D-Ala-D-Ala, a molecule referred to as the **Park peptide** (Fig. 35-2A).

Two more reactions complete the synthesis of a murein monomer. These reactions take place at the inner surface of the cytoplasmic membrane in association with a lipid carrier molecule, **bactoprenol.** Bactoprenol is also used to ferry the completed monomer to the outer surface of the cytoplasmic membrane, where polymerization occurs (Fig. 35-2B). These reactions begin with the **MraY**-mediated transfer of the Park peptide to phosphorylated bactoprenol. This transfer liberates UMP, resulting in a pyrophosphate bond between bactoprenol and the Park peptide. In a reaction catalyzed by the enzyme **MurG,** this new molecule then reacts with a molecule of UDP–NAG, liberating UDP and resulting in the formation of a bond between the NAM of the Park peptide and NAG. Finally, in Gram-positive bacteria, a linker polypeptide, typically composed of five glycine residues, is usually added at the lysine (or DAP) position; as discussed below, this **interbridge** allows polymer crosslinking in Gram-positive bacteria. In Gram-negative bacteria, the murein monomers are usually crosslinked to one another directly, without the use of a linker polypeptide. These steps complete the synthesis of a **murein monomer.**

### Polymerization

The last two phases in cell wall biosynthesis—polymerization (transglycosylation) and crosslinking (transpeptidation)—take place outside the cytoplasm in the periplasmic space. For murein monomers to reach the periplasmic space, they must cross the lipid bilayer of the cytoplasmic membrane. As noted above, this "ferrying" requires bactoprenol. A long, lipophilic molecule, bactoprenol is thought to wrap around the murein monomer, rendering it sufficiently lipophilic to cross the bilayer. Once in the periplasmic space, the monomer is attached to a growing murein chain via bonds between the NAM of the murein monomer and the NAG of the growing peptidoglycan polymer. This transfer, which is catalyzed by transglycosylases, frees bactoprenol pyrophosphate. Bactoprenol pyrophosphate then returns to the cytoplasmic (inner) surface of the cytoplasmic membrane. There, it loses a phosphate group to form bactoprenol phosphate; this step is catalyzed by a **dephosphorylase.** Bactoprenol phosphate is now ready to accept another Park peptide (Fig. 35-2B).

### Crosslinking

In the third and final phase of cell wall synthesis, murein chains are crosslinked to one another by enzymes called **transpeptidases.** Because transpeptidases were first identified as the molecular targets of penicillin binding, they are also called **penicillin-binding proteins** (**PBPs**). The transpeptidase enzyme displaces the terminal D-Ala residue on one peptide chain to form an enzyme-peptidoglycan intermediate; the free

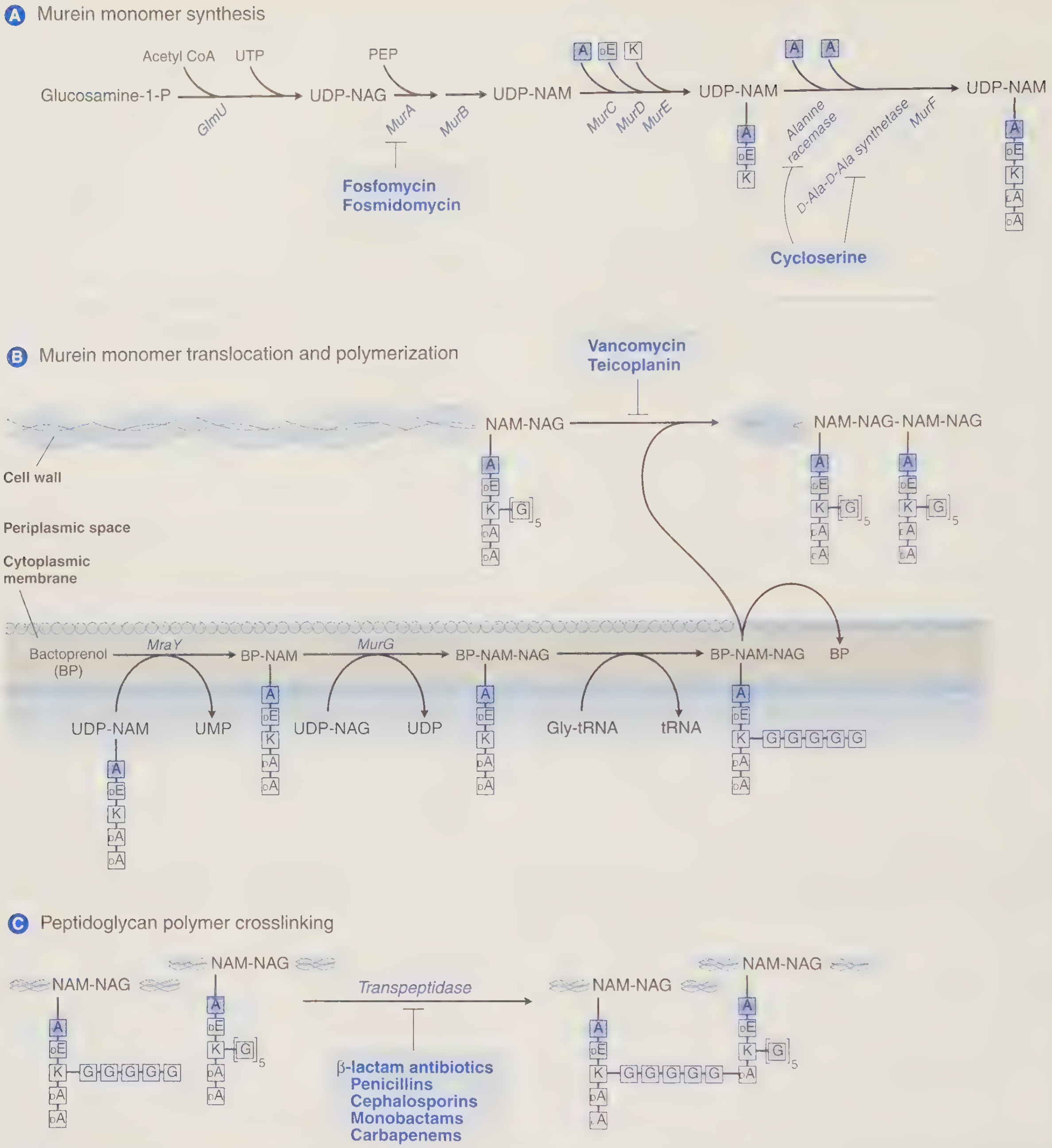

A Murein monomer synthesis
Acetyl CoA
UTP
PEP
Glucosamine-1-P
GlmU
UDP-NAG
MurA
MurB
UDP-NAM
Fosfomycin
Fosmidomycin
A
DE
K
MurC
MurD
MurE
UDP-NAM
Alanine racemase
D-Ala-D-Ala synthetase
MurF
Cycloserine
UDP-NAM
DA
B Murein monomer translocation and polymerization
Vancomycin
Teicoplanin
Cell wall
Periplasmic space
Cytoplasmic membrane
NAM-NAG
NAM-NAG-NAM-NAG
G
5
Bactoprenol (BP)
MraY
BP-NAM
MurG
BP-NAM-NAG
BP-NAM-NAG
BP
UDP-NAM
UMP
UDP-NAG
UDP
Gly-tRNA
tRNA
C Peptidoglycan polymer crosslinking
NAM-NAG
Transpeptidase
β-lactam antibiotics
Penicillins
Cephalosporins
Monobactams
Carbapenems

**Figure 35-2. Bacterial Cell Wall Biosynthesis and Its Inhibition by Pharmacologic Agents.** Bacterial cell wall biosynthesis can be divided into three steps. **A.** In murein monomer synthesis, glucose is amidated and phosphorylated to glucosamine-1-phosphate (not shown), which is acetylated and conjugated to a uridine diphosphate (UDP) nucleotide by the enzyme GlmU to form UDP-*N*-acetylglucosamine (UDP–NAG). Addition of phosphoenolpyruvate (PEP) by enolpyruvate transferase (MurA), and reduction of the resulting product by MurB, forms UDP-*N*-acetylmuramic acid (UDP–NAM). Fosfomycin and fosmidomycin are selective inhibitors of enolpyruvate transferase. NAG and NAM are the two sugar building blocks for subsequent cell wall synthesis. MurC, MurD, and MurE sequentially add the amino acids L-alanine (A), D-glutamate (DE), and L-lysine (K) to UDP–NAM. In some bacteria, diaminopimelic acid (DAP) is added instead of L-lysine. Alanine racemase converts L-alanine to D-alanine (DA), and D-Ala-D-Ala synthetase forms the dipeptide D-Ala-D-Ala. This dipeptide is then added to the A-DE-K (or A-DE-DAP) tripeptide by MurF, resulting in a UDP–NAM molecule linked to five amino acids (Park peptide). Cycloserine inhibits both alanine racemase and D-Ala-D-Ala synthetase, thereby preventing the addition of alanine residues to the growing peptide chain. **B.** The NAM-pentapeptide complex is transferred from UDP to the lipid carrier bactoprenol (BP) by the enzyme MraY, and NAG is added from UDP–NAG by MurG. In some bacteria, one to five amino acids can then be added to K or DAP to form a branched peptidoglycan; the amino acids are added from amino acyl tRNA. (Here, as an example, five glycine (G) residues are added from glycyl-tRNA.) In the murein monomer translocation and polymerization step, the BP-peptidoglycan complex is transported from the bacterial inner membrane to the periplasmic space, where transglycosylases join the murein monomer to the growing peptidoglycan chain. Simultaneously, the BP is liberated to catalzye another round of murein monomer translocation. Completion of this set of reactions depends on serial phosphorylation and dephosphorylation of the bactoprenol molecule (not shown). Bacitracin inhibits bactoprenol dephosphorylation and, thereby, interrupts murein monomer translocation (not shown). Vancomycin and teicoplanin bind the D-Ala-D-Ala terminus of the BP-conjugated murein monomer unit and, thereby, prevent the transglycosidase-mediated addition of the murein monomer to the growing peptidoglycan chain. **C.** In the final step of cell wall biosynthesis, adjacent glycopeptide polymers are crosslinked in a reaction catalyzed by bacterial transpeptidases. In the example shown, a transpeptidase crosslinks a glycine (G) pentapeptide on one peptidoglycan chain to a D-Ala residue on an adjacent peptidoglycan chain; as shown in detail in Figure 35-3, the terminal D-Ala residue is displaced in this reaction. The beta-lactam antibiotics (penicillins, cephalosporins, monobactams, and carbapenems) inhibit the transpeptidase enzymes that crosslink adjacent peptidoglycan polymers.

### BOX 35-1. Enzymes of Cell Wall Biosynthesis

Like most enzymes, the enzymes of cell wall biosynthesis are known by multiple names. The Mur naming convention used here is the emerging standard, but the enzymes are also still known by the following descriptive names (among others):

| | |
|---|---|
| GlmU | diamine N-acetyltransferase |
| MurA | enolpyruvate transferase |
| MurB | UDP–NAG-enolpyruvate reductase |
| MurC | UDP–NAM-L-Ala synthetase |
| MurD | UDP–NAM-L-Ala-D-Glu synthetase |
| MurE | UDP–NAM-L-Ala-D-Glu-2,6-diaminopimelate synthetase |
| MurF | UDP–NAM-tripeptide-D-Ala-D-Ala synthetase |
| MraY | UDP–NAM-pentapeptide:undecaprenylphosphate transferase |
| MurG | undecaprenyldiphospho-NAM-pentapeptide: NAG transferase |

---

Note: Undecaprenol is another name for bactoprenol

amino group on the terminal amino acid of the interbridge peptide (glycine for most Gram-positive bacteria) or on DAP (Gram-negative bacteria) then attacks this intermediate, resulting in formation of the crosslink (Figs. 35-2C, 35-3). Differences in chain length and in the number and type of crosslink give each bacterial species its characteristic shape and size, and the cell wall of each species its characteristic thickness.

Bacteria typically have several transpeptidases of different but overlapping specificities. These different enzyme isoforms are used to build different parts of the wall. *Escherichia coli,* for example, has six transpeptidases, some of which build the cylindrical middle of this rod-shaped bacteria, others of which build its hemispherical ends. Also, the suite of transpeptidases differs from species to species, and especially between rods such as *E. coli* and *C. perfringens* and spherical cocci such as streptococci and staphylococci.

## MYCOBACTERIAL CELL WALL

The cell wall structures described above apply to the vast majority of clinically relevant bacteria, including Gram-positive cocci such as streptococci and staphylococci, Gram-negative rods such as *E. coli* and *Pseudomonas aeruginosa,* and Gram-positive rods such as *C. perfringens.* However, the cell wall of one group of bacteria, the **mycobacteria,** differs in several important respects. Because of the resurgent clinical impact of *Mycobacterium tuberculosis,* the bacterium that causes tuberculosis, it is important to examine the mycobacterial cell wall in some detail.

As with the cell walls of Gram-negative bacteria, the cell wall of mycobacteria consists of a relatively thin layer of murein just outside the cytoplasmic membrane. However, dissimilar to Gram-negative bacteria, in mycobacteria, the NAM residues of the cell wall are modified by the addition of a long branched chain consisting of a NAG-**arabinogalactan** linker topped with **mycolic acid.** Structurally, the lipophilic mycolic acid layer acts as the inner half of an asymmetric outer membrane; the outer half of this membrane is composed of secreted phospholipids, called **extractable lipids,** that are analogous to the lipids that form the outer half of the cytoplasmic membrane (see Fig. 35-1). Overall, then, an analogy can be drawn between the mycobacterial and the Gram-negative cell wall and associated structures. Both mycobacteria and Gram-negative bacteria are enclosed by an inner (cytoplasmic) membrane, a murein (cell wall) layer, and an outer membrane; the main structural difference is that the outer membrane of mycobacteria is thick, asymmetric, and highly impermeable to both hydrophilic and hydrophobic substances.

The synthesis of NAG-arabinogalactan begins with the transfer of a molecule of NAG phosphate from UDP–NAG to mycobacterial bactoprenol phosphate. Next, a molecule of the sugar rhamnose is added, followed by addition of the several galactose and arabinose units that comprise arabinogalactan. Addition of the arabinose units is catalyzed by the enzyme **arabinosyl transferase.**

Mycolic acid is a long, complex, branched fatty acid. The starting materials for its synthesis include a number of long saturated hydrocarbon chains that are synthesized from two-carbon units carried by acetyl CoA. The enzyme **fatty acid synthetase 1** (**FAS1**) catalyzes the formation of these saturated hydrocarbon chains, whereas the enzyme **fatty acid synthetase 2** (**FAS2**) catalyzes the linkage of these chains. The linked product then undergoes several enzymatic transformations to become mycolic acid. Mycolic acid is eventually added to NAG–arabinogalactan, which, in turn, is attached to NAM to form the complete inner half of the mycobacterial outer membrane (Figs. 35-1, 35-4).

In principle, any step in this process is susceptible to pharmacologic intervention. As discussed below, standard antimycobacterial treatment regimens include antibiotics that target both the synthesis of NAG-arabinogalactan and the early reactions of mycolic acid synthesis.

## AUTOLYSINS AND CELL WALL DEGRADATION

For bacteria to grow, bacterial cell walls must expand; for expansion to occur, new murein units must be incorporated into the existing cell wall. This is difficult to accomplish in a "finished" cell wall, where the murein polymer chains are already of the desired length, and there is already the desired type and degree of polymer crosslinking. In addition, for a bacterium to divide into two daughter cells, its cell wall must, at some point, be broken. Bacteria address these issues by using **autolysins.** These enzymes (**NAM-L-alanine amidase,** for example) punch small holes in the cell wall that allow for cell wall remodeling and expansion. Clearly, new murein synthesis and autolysin-mediated destruction must be carefully balanced for the bacteria to survive. Indeed, studies have shown that unilaterally blocking murein synthesis results in autolysin-mediated **autolysis** and cell death. The molecular events that initiate autolysis are still poorly understood. One theory holds that bacterial exposure to antibiotics inhibiting cell wall synthesis leads to leakage and loss of an endogenous autolysin inhibitor, perhaps **lipoteichoic acid** in Gram-positive bacteria, and that this loss, in turn, results in the activation of autolysins and the eventual lysis of the cell. The **bactericidal effect** of many antibiotics discussed

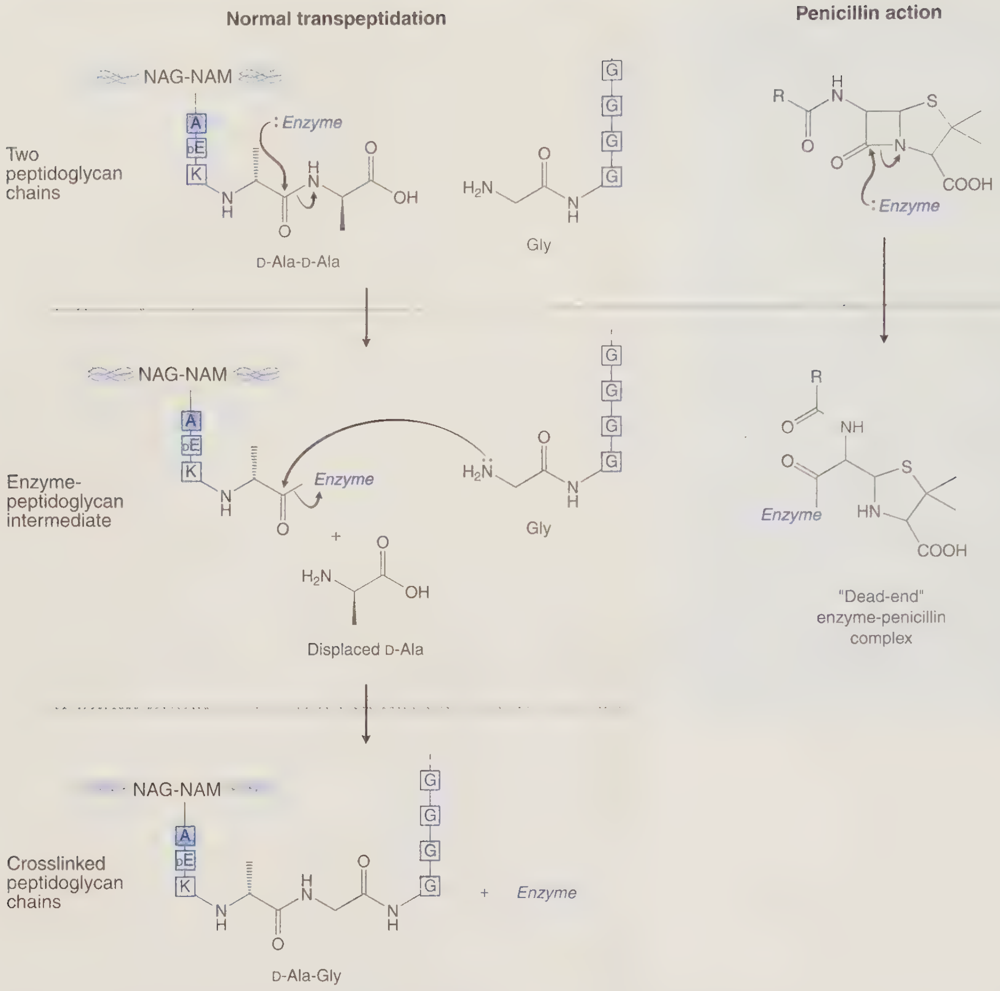

Normal transpeptidation
Penicillin action
NAG-NAM
A
DE
K
Two peptidoglycan chains
: Enzyme
D-Ala-D-Ala
G
Gly
H₂N
OH
R
S
N
COOH
: Enzyme
Enzyme-peptidoglycan intermediate
Enzyme
Displaced D-Ala
Enzyme
"Dead-end" enzyme-penicillin complex
Crosslinked peptidoglycan chains
+ Enzyme
D-Ala-Gly

**Figure 35-3. Transpeptidase Action and Its Inhibition by Penicillin.** The left side of the figure shows the mechanism by which transpeptidases catalyze transpeptidation, a reaction that occurs in bacteria but not in mammalian cells. A nucleophilic group on the transpeptidase (enzyme) attacks the peptide bond between the two D-ala residues at the terminus of a pentapeptide moiety on one peptidoglycan chain (top panel). The terminal D-alanine residue is displaced from the peptidoglycan chain, and an enzyme-D-alanine-peptidoglycan intermediate is formed. This intermediate is then attacked by the amino terminus of a polyglycine pentapeptide linked at its carboxy terminus to L-lysine or diaminopimelic acid on an adjacent peptidoglycan chain (see Fig. 35-2) (middle panel). As the enzyme is liberated from the intermediate, a new peptide bond (crosslink) is formed between the terminal glycine residue on one peptidoglycan chain and the enzyme-activated D-alanine residue on the adjacent peptidoglycan chain. The free enzyme can then catalyze another transpeptidation reaction (bottom panel). The right side of the figure shows the mechanism by which penicillin interferes with transpeptidation, leading to the formation of a penicilloyl-enzyme "dead-end complex." In this form, the enzyme is incapable of catalyzing further transpeptidation (crosslinking) reactions.

in this chapter is thought to result from drug-mediated disruption of the balance between cell wall synthesis and cell wall degradation.

## PHARMACOLOGIC CLASSES AND AGENTS

The pharmacology of the various classes of agents that inhibit bacterial cell wall synthesis is discussed in the same order as the steps inhibited by these drugs (Fig. 35-2). Although drugs have been identified that inhibit a number of steps in the biochemistry of cell wall synthesis, the polymer crosslinking (transpeptidation) step is, by far, the most clinically important biochemical target. For this reason, most of the discussion focuses on the panoply of agents that inhibit the crosslinking of peptidoglycan polymers.

## INHIBITORS OF MUREIN MONOMER SYNTHESIS

### Fosfomycin and Fosmidomycin

Two agents inhibit the production of murein monomers by inhibiting the synthesis of UDP–NAM from UDP–NAG. **Fosfomycin** (also written **phosphomycin**) is a phosphoenolpyruvate (PEP) analogue that inhibits bacterial enolpyru-

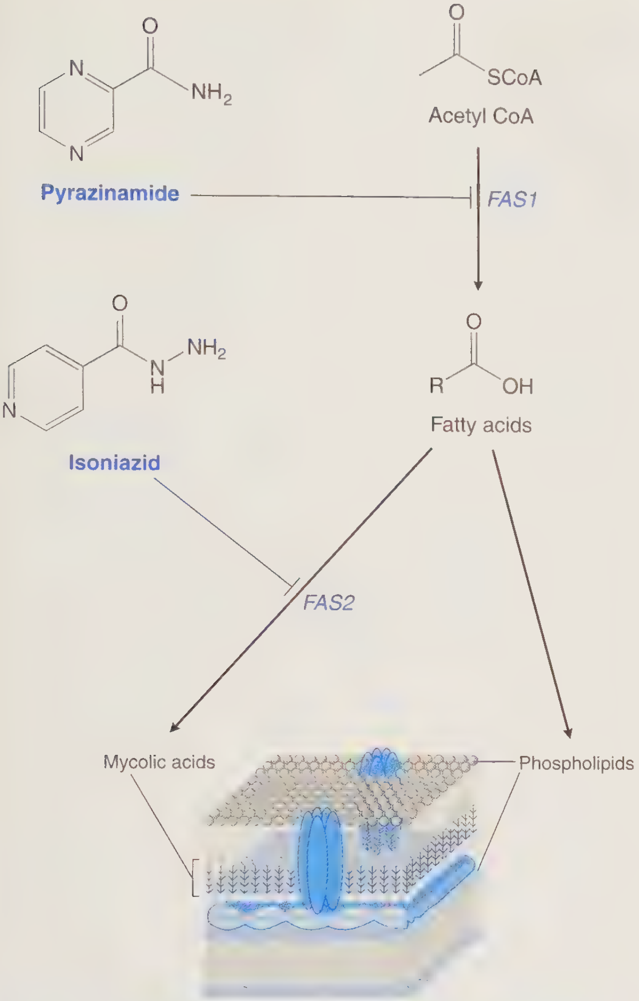


**Figure 35-4. Mycolic Acid Synthesis and Antimycobacterial Drug Action.** Mycolic acids are produced by the crosslinking of fatty acid chains derived from acetyl coenzyme A (Acetyl CoA). Each of the arrows in this simplified representation denotes multiple synthetic steps; the focus is on the fatty acid synthetases (FAS1 and FAS2) because of their importance as drug targets. Specifically, FAS1 is inhibited by pyrazinamide, and FAS2 is inhibited by isoniazid.

vate transferase (also known as MurA) by covalent modification of the enzyme's active site. Given that PEP is a key intermediate in (mammalian) glycolysis, it may come as a surprise that this agent does not interfere with carbohydrate metabolism in human cells; this selectivity of antibacterial action is likely caused by structural differences between the mammalian and bacterial enzymes that act on PEP. Thus, fosfomycin has no appreciable effect on human enolase, pyruvate kinase, or carboxykinase, and the drug is relatively nontoxic. Fosfomycin has been shown to have synergy in vitro with beta-lactams, aminoglycosides, and fluoroquinolones.

Fosfomycin enters the cell through transporters for glycerophosphate or glucose-6-phosphate that are normally used by bacteria to take up these nutrients from the environment. Fosfomycin is especially effective against Gram-negative bacteria that infect the urinary tract, including *E. coli* and *Klebsiella* and *Serratia* species because it is excreted unchanged in the urine. As a rule, fosfomycin is less effective against Gram-positive bacteria because these bacteria generally lack selective glycerophosphate and glucose-6-phosphate transporters. Although resistance is typically caused by mutations in these transporters, a temperature-sensitive *E. coli* strain has been found in which a mutation in enolpyruvate transferase results in reduced affinity of the enzyme for PEP and, therefore, for fosfomycin.

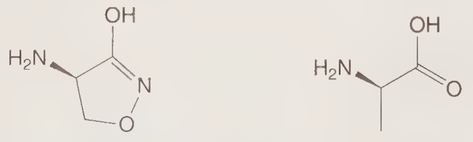
**Fosmidomycin,** another PEP analogue, acts by the same mechanism as fosfomycin, and resistance typically arises via mutations in glycerophosphate or glucose-6-phosphate transporters. Again, however, there are exceptions: at least one strain of resistant *E. coli* seems to contain a protein that actively pumps fosmidomycin out of the cell. Fosmidomycin also has activity against malaria, but the drug has a different mechanism of action against that parasite.

## Cycloserine

**Cycloserine,** a structural analogue of D-Ala, is a second-line agent against *M. tuberculosis* infection (Fig. 35-5). Although many species have become resistant to cycloserine since its introduction some 50 years ago, the drug remains active against a number of Gram-positive and Gram-negative bacteria. Cycloserine inhibits both the alanine racemase that converts L-Ala to D-Ala and the D-Ala-D-Ala synthetase that joins together two D-Ala molecules (Fig. 35-2). Cycloserine is an irreversible inhibitor of these enzymes and, in fact, binds these enzymes more tightly than does their natural substrate, D-Ala. Resistance occurs by multiple mechanisms, some of which are still unknown; known mechanisms include the overexpression of alanine racemase and mutations in the alanine uptake system. As with many small molecules, including fosfomycin, cycloserine is excreted in the urine. Its chief side effects are psychosis and peripheral neuropathy; the peripheral neuropathy can, in some cases, be lessened by coadministration of pyridoxine.

## Bacitracin

So named because it was first identified in a species of *Bacillus,* **bacitracin** is a peptide antibiotic that interferes with the dephosphorylation of bactoprenol pyrophosphate, rendering this lipid carrier useless for further rounds of murein monomer translocation (Fig. 35-2). Bacitracin is, thus, notable among the anticell wall agents for having a lipid, rather than a protein or peptide, as its target. Bacitracin inhibits dephosphorylation by forming a complex with bactoprenol pyrophosphate that involves bacitracin's imidazole and thiazoline rings.



**D-Cycloserine** D-Alanine

**Figure 35-5. Structure of Cycloserine.** Cycloserine is a structural analogue of D-alanine that inhibits the racemic interconversion of L-alanine to D-alanine by alanine racemase. Cycloserine also inhibits the activity of D-Ala-D-Ala synthetase, the enzyme that catalyzes the formation of the D-Ala-D-Ala dipeptide that is subsequently utilized in the synthesis of murein monomers (see Fig. 35-2).

This interaction requires a divalent metal ion, usually $Zn^{2+}$ or $Mg^{2+}$; hence, drugs that act as metal chelators could be predicted to interfere with the activity of bacitracin. The nephrotoxicity associated with systemic administration of bacitracin restricts the use of this drug to topical applications, except, in unusual cases, against resistant *C. difficile* infections.

## INHIBITORS OF MUREIN POLYMER SYNTHESIS

### Vancomycin and Teicoplanin

**Vancomycin** and **teicoplanin** are glycopeptides with bactericidal activity against Gram-positive rods and bacteriostatic activity against Gram-positive cocci. Gram-negative rods are resistant to the action of these drugs. These agents interrupt cell wall synthesis by binding tightly to the D-Ala-D-Ala terminus of the murein monomer unit, inhibiting **transglycosidase** and, thereby, blocking the addition of more murein units to the growing polymer chain. Intravenous vancomycin is most commonly used to treat sepsis or endocarditis caused by methicillin-resistant *Staphylococcus aureus* (see below). Oral vancomycin is used against gastrointestinal infections with *Clostridium difficile* because the drug is poorly absorbed and, therefore, stays within the gastrointestinal tract.

As a rule, the toxicity and side effects of vancomycin cause this agent to be used only when an infection is found to be resistant to other agents. Its toxicity includes skin flushing or rash—the so-called **red neck** (or **red man**) **syndrome**—which can be avoided by slowing the rate of intravenous infusion or preadministering antihistamines. Teicoplanin shares this toxicity. Vancomycin has occasionally been associated with nephrotoxicity and ototoxicity, but nephrotoxicity is typically a serious risk only in patients to whom aminoglycosides are coadministered with vancomycin.

Resistance to vancomycin most commonly arises through the acquisition of an enzyme that catalyzes the formation of D-Ala-D-lactate instead of D-Ala-D-Ala. As with D-Ala-D-Ala, D-Ala-D-lactate is incorporated into the murein monomer unit and participates readily in the transpeptidase reaction, but the D-Ala-D-lactate dipeptide is not bound by vancomycin. The enzyme responsible for vancomycin resistance, called VanA, is encoded on a transposable element that can be found on either the bacterial chromosome or an extrachromosomal plasmid. In clinical practice, bacteria resistant to vancomycin (such as **vancomycin-resistant enterococci**) are almost always resistant to all other antibacterials; plasmid-mediated spread of vancomycin resistance is, therefore, a serious medical problem.

## INHIBITORS OF POLYMER CROSSLINKING

### Beta-Lactam Antibiotics: General Considerations

With more than thirty different agents currently in use, including the original **penicillin** used in the attempt to treat Pvt. Harris, the beta-lactams are the largest and most widely prescribed class of antibiotics that inhibit bacterial cell wall synthesis. The different agents in this class vary in chemical structure (Fig. 35-6) and, consequently, in spectrum of action, but all beta-lactams share the same antibiotic mechanism of action: inhibition of murein polymer crosslinking.

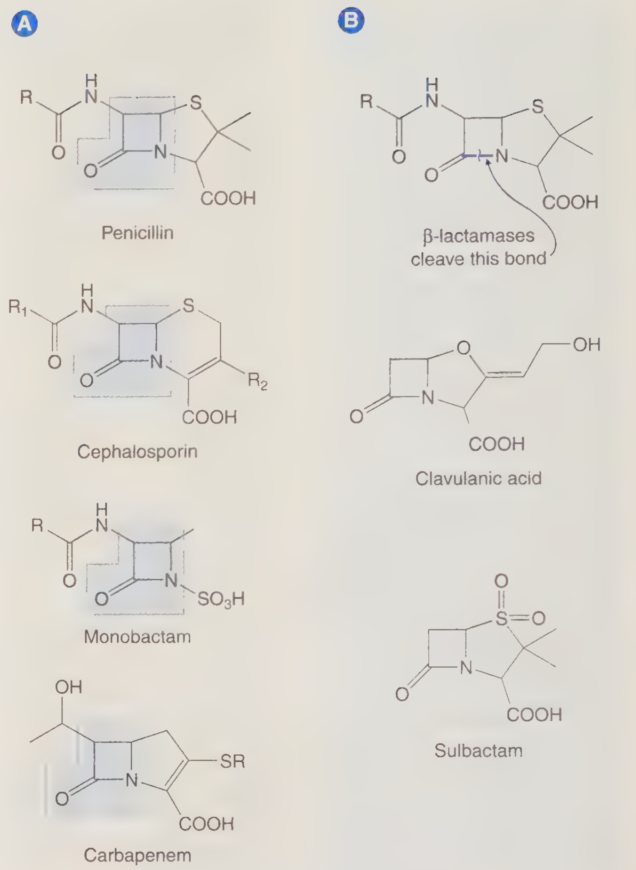


Figure 35-6. Structural Features of Beta-Lactam Antibiotics and Beta-Lactamase Inhibitors. **A.** The beta-lactam family members (penicillins, cephalosporins, monobactams, and carbapenems) differ from one another in their backbone structures; individual drugs within these subclasses also differ in their R groups. Note the four-membered beta-lactam ring that is common to all four families (blue boxes); it is this ring that gives the agents their ability to block the transpeptidation reaction (and also their name). **B.** Bacteria expressing beta-lactamases are able to cleave the beta-lactam bond (blue line) that is required for antibiotic action. The beta-lactamase inhibitors clavulanic acid and sulbactam act as decoys by binding to (and, thereby, inhibiting) beta-lactamase enzymes. Note the structural similarity between the beta-lactamase inhibitors and the beta-lactam antibiotics.

Chemically, the key to this mechanism of action is the presence, in all beta-lactams, of a four-membered **beta-lactam ring** (Fig. 35-6). This ring makes every beta-lactam a structural analogue of the terminal D-Ala-D-Ala dipeptide of the Park peptide and, hence, a substrate for one or several bacterial transpeptidases. As with the Park peptide, the beta-lactam is able to bind the transpeptidase covalently and, thereby, form an acyl enzyme intermediate. However, unlike the Park peptide in the normal substrate reaction, the beta-lactam ring renders the carboxy terminal end of the beta-lactam unable to be cleaved from the rest of the molecule. As a result, the incoming amino terminal end of the adjacent peptide cannot attack the acyl enzyme intermediate, and the transpeptidase reaches a **"dead-end" complex** (Fig. 35-3). (This mode of irreversible enzyme inhibition is sometimes called **suicide substrate inhibition.**) Provided that the cells are growing, transpeptidase inhibition results in autolysin-mediated autolysis and cell death. Hence, as a rule, beta-lactams are **bactericidal** for actively dividing bacteria.

The different subclasses of beta-lactam agents fall into four families—the **penicillins,** the **cephalosporins** (which are further subdivided into four "generations"), the **monobactams,** and the **carbapenems.** Each of these subclasses differs structurally in the chemical substituents that are attached to the beta-lactam ring (see Fig. 35-6). In general, these families resulted from pharmacologists's efforts in the laboratory to improve on penicillin's antibiotic **spectrum of action** and to keep ahead of the spread of **antibiotic resistance,** as was seen in the case of Pvt. Harris. (Recall that spectrum of action refers to the number and variety of bacterial species against which an antibiotic shows bactericidal or bacteriostatic activity. Hence, broad spectrum beta-lactams are typically active against Gram-negative as well as Gram-positive bacteria; whereas narrow spectrum beta-lactams are typically effective only against Gram-positive organisms.)

Because bacterial transpeptidases are located in the periplasmic space between the cytoplasmic membrane and the cell wall, beta-lactams must traverse the cell wall, and, in the case of Gram-negative bacteria, the outer membrane, to exert their effect. Hence, a beta-lactam's spectrum of action is determined by two factors: the degree to which it can penetrate the outer membrane and cell wall and, once in the periplasmic space, its ability to bind to specific transpeptidases. Both hydrophilic and (to a lesser extent) hydrophobic agents diffuse through the thick murein layer of Gram-positive bacteria, but hydrophilic agents pass through the outer membrane pores of Gram-negative bacteria more readily than do hydrophobic agents. As a result, such hydrophilic agents as **ampicillin, amoxicillin,** and especially **pipericillin, ticarcillin, carbenicillin,** and **mezlocillin** tend to have broad spectra of action, whereas such hydrophobic agents as **oxacillin, cloxacillin, dicloxacillin, nafcillin, methicillin,** and **penicillin G**—a close relative of the agent that was available to soldiers during the Korean War—tend to have narrow spectra of action (see below for details). This means that some Gram-negative bacteria are inherently resistant to narrow spectrum beta-lactams simply by virtue of the permeability barrier presented by their outer membranes. (Similarly, **intracellular bacteria**—that is, bacteria that live within human cells—are, in general, also inherently resistant to beta-lactams, both because mammalian cells tend to lack beta-lactam uptake mechanisms and because these bacteria tend either to have unique cell wall architectures or to lack cell walls altogether.)

The second factor that determines a beta-lactam's spectrum of action is the extent to which the drug, after accessing the periplasmic space, can inhibit a particular transpeptidase. This is determined, in large part, by the beta-lactam's affinity for the transpeptidase. As noted above, bacteria typically have several transpeptidases that differ subtly in their substrate specificity and crosslinking activity; these differences are especially prominent between rods and cocci. Most beta-lactams have specificity for several different transpeptidases; others, such as the penicillin methicillin that is used mainly against *Staphylococcus aureus,* are specific for just one.

Antibiotic resistance can be either **chromosomal** (**intrinsic**) or **acquired** (**extrinsic**). For beta-lactams, chromosomal resistance is most commonly conferred by a mutation in a transpeptidase-encoding gene that abolishes the transpeptidase's ability to bind a particular beta-lactam. This is the mechanism by which *S. aureus* acquires resistance to methicillin, and the mechanism by which pneumococci acquire resistance to penicillin. However, chromosomal resistance to beta-lactams is the exception, not the rule. This is because most beta-lactams are active against multiple transpeptidases and, therefore, multiple coexisting mutations would be required to abolish their effectiveness; hence, chromosomal resistance is rare.

Most resistance to beta-lactams is acquired, and is conferred by proteins called **beta-lactamases** that are encoded on extrachromosomal DNA **plasmids.** Acquisition of such a plasmid is probably the mechanism by which resistance emerged in the *C. perfringens* that infected Pvt. Harris. As their name implies, beta-lactamases are enzymes that inactivate beta-lactams via (hydrolytic) cleavage of the beta-lactam ring. More than 100 different beta-lactamases have been identified, each with activity against a particular beta-lactam or set of beta-lactams. Beta-lactamases are secreted in Gram-positive bacteria, whereas in Gram-negative bacteria, these enzymes are retained in the periplasmic space between the cell wall and outer membrane. The latter organisms produce much less beta-lactamase than the former, but because they concentrate the beta-lactamase where it is needed, the beta-lactamase is more effective at conferring resistance. This concentration effect, coupled with the strong permeability barrier to penicillins afforded by the bacterial outer membrane, makes Gram-negative bacteria largely refractory to penicillin therapy.

That beta-lactamases are encoded on plasmids is of special clinical importance. Because plasmids are easily transferred by conjugation from one bacterium to another, the resistance conferred by the plasmid can sweep rapidly through a bacterial population. Moreover, plasmids can "jump strains," spreading resistance from one strain to another. Pharmacologists' response has been twofold. First, as noted above, has been the development of new families of beta-lactams whose structures make them less susceptible to cleavage by existing beta-lactamases. Second has been the coadministration of beta-lactams with **beta-lactamase inhibitors,** which are beta-lactam-like molecules that bind to beta-lactamases and, thereby, keep the beta-lactamases from destroying the beta-lactams with which the lactamase inhibitors are coadministered. In general, broad spectrum beta-lactams are beta-lactamase sensitive, whereas narrow spectrum beta-lactams are beta-lactamase resistant. Hence, beta-lactamase-sensitive beta-lactams—such as pipericillin, ticarcillin, carbenicillin, mezlocillin, and especially ampicillin and amoxicillin—are frequently coadministered with beta-lactamase inhibitors. Two examples of beta-lactamase inhibitors are **clavulanic acid** (**clavulanate**) and the **sulbactams** (e.g., **tazobactam**) (Fig. 35-6).

Beta-lactams act synergistically with **aminoglycosides,** the bactericidal inhibitors of protein synthesis discussed in Chapter 33. (For more on synergy, see Chapter 38, Principles of Combination Chemotherapy.) Aminoglycosides inhibit protein synthesis by binding to the 30S ribosomal subunit in the cytoplasm of the cell. To access the cytoplasm, aminoglycosides must diffuse passively across the cell wall before being transported actively across the cytoplasmic membrane. It is thought that the cell walls of some bacteria, such as enterococci, are essentially impermeable to aminoglycosides when these drugs are administered as single agents. Because the beta-lactams act to increase cell wall permeability, coadministration of a beta-lactam facilitates the uptake of an aminoglycoside and, thus, enhances its effect.

One could wonder whether the aminoglycosides reciprocate by potentiating the activity of beta-lactams, or whether, instead, the aminoglycosides antagonize beta-lactams by inhibiting the synthesis of autolysins. From work on *Bacillus subtilis,* it seems that bacterial cell walls contain a lethal amount of autolysins throughout cell growth, and that cells actively restrain autolytic activity by controlling the state of activation of these proteins. This finding suggests that autolysis does not require de novo autolysin synthesis and, therefore, that aminoglycosides should not antagonize beta-lactams. Regardless, the important point is that *clinically, beta-lactams and aminoglycosides are synergistic.*

The most common toxic side effects of beta-lactam therapy are those related to hypersensitivity reactions. As small molecules, beta-lactams are not expected to stimulate immune responses by themselves, and indeed they do not. However, beta-lactam rings can react with amino groups on proteins and, in the process, make these proteins antigenic (Fig. 35-7). The beta-lactam-protein conjugate can then provoke an immune response, which is most commonly manifested as a drug rash but can occasionally be much more severe. Modification of proteins on the surface of red blood cells, leading to antibody-mediated hemolysis, is another example of this effect. For most individuals, this process is strongly dose-dependent: the likelihood of a hypersensitivity reaction increases with each administration of a beta-lactam. Beta-lactams of a given class often cross-react with each other, but beta-lactams of one class are typically much less cross-reactive with beta-lactams of another class. For example, ampicillin should ordinarily not be used in a penicillin-sensitive patient, whereas cephalosporins and monobactams generally could be used in such a patient.

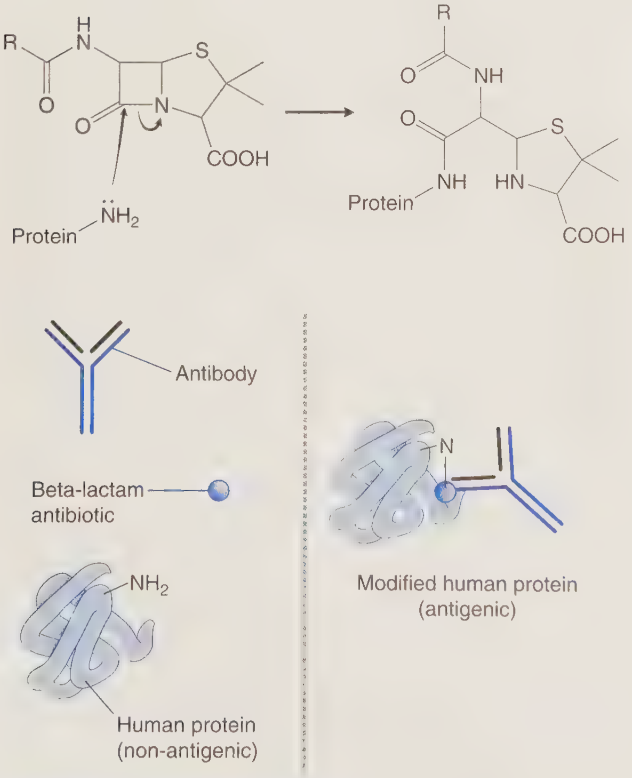


**Figure 35-7. Beta-Lactam Toxicity. Upper panel:** Beta-lactams can modify amino groups on human proteins, creating an immunogenic beta-lactam hapten. **Lower panel:** In the absence of modification, human proteins are nonantigenic. Modification of endogenous proteins by the addition of a beta-lactam antibiotic results in the formation of a new antigenic determinant that can be recognized as "foreign" by antibodies of the host immune system.

## Beta-Lactam Antibiotics: Specific Agents

### Penicillins

As noted above, there are four structurally distinct subclasses of beta-lactam antibiotics (see Fig. 35-6A). The first of these subclasses, the penicillins, can be further divided into four groups according to their spectra of action.

The first group of penicillins includes penicillin G, which is intravenously administered, and **penicillin V,** its oral counterpart. Despite the spread of resistance, penicillin G is still active against such Gram-positive bacteria as pneumococcus and *S. pyogenes* (some strains of each), the Gram-negative coccus *Neisseria meningitidis,* Gram-positive rods of the *Clostridia* genus, and spirochetes such as syphilis and *Leptospira.* Penicillin G is generally preferred over penicillin V in the inpatient setting, because intravenous administration is both more effective and more convenient in the hospital.

Members of the second group of penicillins are active predominantly against Gram-positive cocci; in practice, these agents are used chiefly against *Staphylococcus aureus.* The **antistaphylococcal penicillins** include oxacillin, cloxacillin, dicloxacillin, nafcillin, and methicillin. These drugs are structurally resistant to beta-lactamases, but, in part, because of their relative hydrophobicity, have a narrow spectrum of action. (Recall that methicillin, for example, binds to only a single transpeptidase.) Methicillin's utility as an effective second- or third-line agent against *S. aureus* has made the emergence of methicillin-resistant *S. aureus* strains (known by the abbreviation **MRSA**) a major clinical problem. When a case of MRSA is found in the hospital, special precautions are taken to prevent its spread. In practice, MRSA is often already resistant to other beta-lactams and is typically treated with vancomycin.

Ampicillin and amoxicillin are members of the third group of penicillins. These agents have a broad spectrum of action but are beta-lactamase sensitive. Accordingly, when coadministered with such a beta-lactamase inhibitor as clavulanic acid or sulbactam, these agents are effective against a variety of Gram-positive cocci, Gram-negative cocci such as *Neisseria gonorrhoeae* and *N. meningitidis,* and Gram-negative rods such as *E. coli* and *H. influenzae.* Usually, the penicillin and beta-lactamase inhibitor are combined in a single tablet. The combination of amoxicillin and clavulanic acid is available under the trade name **Augmentin,** and ampicillin–sulbactam is available as **Unasyn.**

Agents in the fourth group of penicillins are also broad in spectrum, but these drugs are reserved chiefly for use against *Pseudomonas aeruginosa,* a dangerous community-

or hospital-acquired bacterium. These **antipseudomonal penicillins** include carbenicillin, piperacillin, ticarcillin, and mezlocillin. As with amoxicillin, the antipseudomonal penicillins are beta-lactamase sensitive, and are often co-administered with clavulanic acid.

### *Cephalosporins*

Cephalosporins differ structurally from penicillins by having a six-membered rather than a five-membered accessory ring attached to the beta-lactam ring. The first-generation cephalosporins **cefazolin** and **cephalexin** are active against Gram-positive species, but are also useful against the Gram-negative rods *Proteus mirabilis* and *E. coli,* both of which cause urinary tract infections, and *Klebsiella pneumonia,* which causes pneumonia. These agents are beta-lactamase sensitive. The second-generation cephalosporins **cefuroxime, cefotetan, cefoxitin,** and **cefamandole** have broader Gram-negative coverage and are useful against *H. influenzae* and *Neisseria* species as well as *Proteus, E. coli,* and *Klebsiella.* Also, these drugs are generally more resistant to beta-lactamases than are the first-generation cephalosporins. Third- (**ceftazidime, ceftriaxone, cefizoxime,** and **cefotaxime**) and fourth-generation cephalosporins include hydrophobic agents that are useful against infections of the central nervous system, as well as some agents that are active against *Pseudomonas aeruginosa.*

As noted above, cephalosporins can generally be used in penicillin-sensitive patients; nevertheless, cephalosporins can cause hypersensitivity reactions themselves and should be avoided in cephalosporin-sensitive patients. Three cephalosporins have side effects that deserve special mention. Cefamandole, cefotetan, and cefoperazone cause an alcohol intolerance syndrome known as the **disulfiram-like reaction** (disulfiram is a drug that inhibits alcohol metabolism; see Chapter 16, Pharmacology of Chemical Dependance and Addiction). These drugs also block the synthesis of vitamin K-dependent clotting factors and should be used with caution in patients taking warfarin and in patients with underlying clotting abnormalities (see Chapter 21, Pharmacology of Hemostasis and Thrombosis). Ceftotetan can also cause antibody-mediated hemolysis.

### *Monobactams and Carbapenems*

The monobactam **aztreonam** is active against nearly all Gram-negative bacteria, but this drug has no Gram-positive coverage. Its use is limited by the side effects of fever and phlebitis.

There are two carbapenems used in clinical practice: **imipenem** and **meropenem.** Both give broad Gram-positive and Gram-negative coverage, although neither is active against MRSA or *Legionella.* Because imipenem is inactivated by the renal enzyme dihydropeptidase I, this drug is always coadministered with the dihydropeptidase inhibitor **cilastatin.** Meropenem has slightly broader Gram-negative coverage than imipenem, is not inactivated by dihydropeptidase, and does not require cilastatin coadministration. Both agents can cause hypersensitivity reactions and, at high plasma drug levels, both can cause seizures.

## ANTIMYCOBACTERIAL AGENTS: ETHAMBUTOL, PYRAZINAMIDE, AND ISONIAZID

**Ethambutol, pyrazinamide,** and **isoniazid** are three of the five front-line agents used to fight infection with tuberculosis (rifampin and streptomycin, discussed in Chapter 33, are the other two). Ethambutol decreases arabinogalactan synthesis by inhibiting the arabinosyltransferase that adds arabinose units to the growing arabinogalactan chain. Ethambutol has been shown to be mycostatic. The other two agents decrease mycolic acid synthesis. Pyrazinamide has recently been shown to inhibit FAS1, the enzyme that synthesizes the fatty acid precursors of mycolic acid. Isoniazid and the related second-line agent **ethionamide** target the FAS2 complex and are mycocidal, although the exact mechanism of action is unknown. The targets of the antimycobacterials are summarized in Figure 35-4.

Antimycobacterial agents are almost always used in combination. A powerful argument in favor of combination therapy in tuberculosis is based on the frequency of resistance mutations and the number of bacteria present in a clinical infection. Each tuberculous lesion in an infected lung can contain $10^8$ bacteria. The frequency of mutants resistant to any single antimycobacterial drug is about one in $10^6$ bacteria. This means that, in each tuberculous lesion, an average of about 100 bacteria will already be resistant to an antimycobacterial drug, even before that drug is administered. Combination therapy with just two drugs reduces the likelihood of encountering pre-existing resistance to just one bacterium in $10^{12}$; treatment with four drugs lowers this probability to one in $10^{24}$ (see Chapter 38). Although these numbers were not yet available when Dr. Pierce put in his request for isoniazid, he knew from qualitative analysis that he could maximize his patients' chances for survival and recovery from tuberculosis by combining streptomycin with isoniazid, a selective antimycobacterial agent that had been introduced in 1952.

Antimycobacterial agents are associated with a number of idiosyncratic toxicities. Ethambutol is associated with optic neuritis and loss of visual acuity; patients taking this drug should receive frequent eye examination. Pyrazinamide is associated with arthralgias and (usually asymptomatic) hyperuricemia. Isoniazid is associated with hepatitis and peripheral neuropathy. Isoniazid also inhibits the liver's cytochrome P450 system and, thereby, interacts with a number of other drugs, including rifampin, the antiseizure medications carbamazepine and phenytoin, azole-type antifungals, and alcohol.

Resistance to these drugs, and to antimycobacterial agents in general, is typically chromosomal. Ethambutol resistance most often results from mutations in, or overexpression of, target enzymes, whereas resistance to isoniazid usually results from a mutation in the mycobacterial enzyme **catalase-peroxidase,** which must activate isoniazid for the drug to have its antimycobacterial effect.

## ■ *Conclusion and Future Directions*

The bacterial cell wall presents a number of unique targets to clinicians and pharmacologists. This structure consists of a three-dimensional mat of crosslinked peptide–sugar polymers called murein and is synthesized in three phases: (1) synthesis of murein monomers; (2) polymerization of monomers into murein polymers; and (3) crosslinking of polymers to complete the wall.

Antibacterial agents act in all three phases of cell wall synthesis: fosfomycin and cycloserine act in the first phase; vancomycin, teicoplanin, and bacitracin act in the second phase; and the beta-lactams, the largest and most important group, act in the third phase. Beta-lactams—which include the penicillins, cephalosporins, monobactams, and carbapenems—are bactericidal; autolytic cell death most likely results from the unopposed action of wall remodeling proteins, called autolysins. Structural and chemical differences among the beta-lactams determine their spectrum of activity against bacteria with different cell wall architectures.

Resistance to beta-lactam antibiotics is generally conferred by plasmid-encoded beta-lactamases. Pharmacologists have addressed this mechanism of resistance: (1) by developing new beta-lactam agents; for example, the second- and third-generation cephalosporins that cannot be cleared (destroyed) by known beta-lactamases; and (2) by coadministering beta-lactam "decoys," such as clavulanic acid and sulbactam, that serve as beta-lactamase inhibitors. Because resistance to beta-lactams is encoded on plasmids, it spreads through bacterial (and human) populations with great speed, making antibiotic development an ongoing arms race. Antimycobacterial agents act by blocking various steps in the synthesis of molecules, such as mycolic acid and arabinogalactan, that are unique to the mycobacterial cell wall. Resistance to these agents is typically chromosomal, but combination therapy is critically important for avoiding the spread of resistance. Future innovations will likely include the development of new agents directed against the myriad unique molecular targets that are presented by the biochemistry of the bacterial cell wall.

## *Suggested Readings*

Brennan PJ. The envelope of mycobacteria. Ann Rev Biochem 1995;64:29–63. (*Review of the structure, composition, and synthesis of the mycobacterial cell wall.*)

El Zoeiby A, Sanschagrin F, Levesque RC. Structure and function of the Mur enzymes: development of novel inhhibitors. Mol Microbiol 2003;47:1–12. (*Current review of the structure, catalytic action, and inhibition of MurA-MurF.*)

Gale EF, Cundliffe E, Reynolds PE, et al. The Molecular Basis of Antibiotic Action. 2nd ed. London: John Wiley; 1981. (*Classic on antibiotics that describes the experiments that led to the determination of many of the mechanisms of action discussed in this chapter.*)

Green DW. The bacterial cell wall as a source of antibacterial targets. Expert Opin Ther Targets 2002;6:1–19. (*Up-to-date discussion of target-based strategies for the development of new antibacterial drugs that inhibit cell wall synthesis.*)

Madigan MT, Martingo JM, Parker J, eds. Brock's Biology of Microorganisms. 9th ed. New York: Prentice-Hall; 1999. (*Chapter 3 presents a good general introduction to bacterial cell wall structure.*)

Rattan A, Kalia A, Ahmad N. Multidrug-resistant *Mycobacterium tuberculosis:* molecular perspectives. Emerg Infect Dis 1998; 4:195–209. (*Discussion of the problem of resistance in tuberculosis.*)

Smith TJ, Blackman SA, Foster SJ. Autolysins of *Bacillus subtilis:* multiple enzymes with multiple functions. Microbiol 2000;146: 249–262. (*Current review of autolysis.*)

## Drug Summary Table Pharmacology of the Bacterial Cell Wall

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF MONOMER SYNTHESIS**<br>*Mechanism—Various; see Notes* | | | | |
| **Fosfomycin**<br>**Fosmidomycin** | Gram-negative urinary tract infections: *E. coli, Klebsiella, Serratia, Clostridia* | | | Structural PEP analogues<br>Inhibit enolpyruvate transferase<br>Synergistic with beta-lactams, aminoglycosides, and fluoroquinolones<br>Excreted in urine |
| **Cycloserine** | *M. tuberculosis* (second line) | Psychosis<br>Peripheral neuropathy | | Structural D-Ala analogue<br>Inhibits alanine racemase and D-Ala-D-Ala ligase |
| **Bacitracin** | Topical infection with *C. difficile* (rarely) | Nephrotoxicity | | Inhibits dephosphorylation of bactoprenol pyrophosphate |
| **INHIBITORS OF POLYMERIZATION**<br>*Mechanism—Inhibit transglycosidase* | | | | |
| **Vancomycin**<br>**Teicoplanin** | MRSA sepsis (IV)<br>*C. difficile* (oral) | Red man syndrome<br>Nephrotoxicity | Increased nephrotoxicity with aminoglycosides | Often used against MRSA |
| **INHIBITORS OF CROSSLINKING: PENICILLINS**<br>*Mechanism—β-lactam structure inhibits transpeptidases* | | | | |
| **Penicillin G**<br>**Penicillin V** | *S. pyogenes* (some strains), *N. meningitidis, Clostridia* spp., syphilis, leptospiridium | All penicillins can cause hypersensitivity reactions (cross-reactive with all penicillins) | | Intravenous penicillin G is preferred to oral penicillin V in hospital settings<br>Beta-lactamase sensitive |
| **Oxacillin**<br>**Cloxacillin**<br>**Dicloxacillin**<br>**Nafcillin**<br>**Methicillin** | *S. aureus* | | | MRSA is generally treated with vancomycin<br>Narrow spectrum<br>Beta-lactamase resistant |
| **Ampicillin**<br>**Amoxicillin**<br>**Amoxicillin/clavulanic acid**<br>**Ampicillin/sulbactam** | Gram-positive cocci, *Neisseria* spp., *E. coli, H. influenza* | | | Broad spectrum<br>Beta-lactamase sensitive as single agents<br>Clavulanic acid and sulbactam are beta-lactamase inhibitors |
| **Carbenicillin**<br>**Piperacillin**<br>**Ticarcillin**<br>**Mezlocillin** | *P. aeruginosa* | | | Broad spectrum<br>Beta-lactamase sensitive |

*(Continued)*

**Drug Summary Table** **Pharmacology of the Bacterial Cell Wall** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF CROSSLINKING: CEPHALOSPORINS**<br>*Mechanism—β-lactam structure inhibits transpeptidases* | | | | |
| **Cefazolin**<br>**Cephalexin** | *Proteus mirabilis, E. coli, Klebsiella pneumoniae* | Hypersensitivity reactions (rarely cross-reactive with penicillins) | | 1st generation agents<br>Relatively good Gram-positive coverage<br>Beta-lactamase sensitive |
| **Cefuroxime**<br>**Cefotetan**<br>**Cefoxitin** | *H. influenza, Enterobacter* spp., *Neisseria* spp., *P. mirabilis, E. coli, K. pneumoniae* | Hypersensitivity reactions (rarely cross-reactive with penicillins) | | 2nd generation agents<br>Relatively stronger Gram-negative coverage<br>More beta-lactamase resistant |
| **Ceftazidime**<br>**Cefoperazone**<br>**Cefamandole**<br>**Ceftriaxone**<br>**Ceftizoxime**<br>**Cefotaxime** | Ceftazidime: *P. aeruginosa*<br>Ceftriaxone: *N. gonorrheae, Borrelia burgdorferi*<br>Cefotaxime, ceftriaxone: *H. influenza* | Cefamandole, cefoperazone: disulfiram-like reaction with alcohol ingestion, block vit. K-dependent clotting factors<br>Hypersensitivity reactions (rarely cross-reactive with penicillins) | | 3rd generation agents<br>Highest CNS penetration of the cephalosporins |
| **INHIBITORS OF CROSSLINKING: MONOBACTAMS / CARBAPENEMS**<br>*Mechanism—β-lactam structure inhibits transpeptidases* | | | | |
| **Aztreonam** | Gram-negative species | Fever<br>Phlebitis | | Monobactam<br>No Gram-positive coverage |
| **Imipenem/cilastatin**<br>**Meropenem** | Gram-positive and Gram-negative species except MRSA and *Legionella* | Seizures at high levels<br>Hypersensitivity reactions | | Cilastatin inhibits renal dihydropeptidase I, which would otherwise inactivate imipenem<br>Meropenem has broader Gram-negative coverage and does not require cilastatin |
| **ANTIMYCOBACTERIAL AGENTS**<br>*Mechanism—Various, see Notes* | | | | |
| **Ethambutol** | Mycobacterium spp. | Optic neuritis<br>Loss of visual acuity | | Inhibits arabinosyl transferase<br>Mycobacteriostatic<br>Used in combination with other antimycobacterials, including rifampin and streptomycin |
| **Pyrazinamide** | Mycobacterium spp. | Arthralgias<br>Hyperuricemia (usually asymptomatic) | | Inhibits fatty acid synthase 1 (FAS1)<br>Used in combination with other antimycobacterials, including rifampin and streptomycin |
| **Isoniazid**<br>**Ethionamide** | Mycobacterium spp. | Isoniazid: hepatitis, peripheral neuropathy, CYP450 interactions | | Inhibit fatty acid synthase 2 (FAS2)<br>Mycobactericidal<br>Used in combination with other antimycobacterials, including rifampin and streptomycin |

# 36

# Pharmacology of Fungal Infections

Lorne W. Murray and Robert H. Rubin

**Introduction**
**Case**
**Biochemistry of Fungal Membranes and Cell Walls**
**Pathophysiology of Fungal Infections**
**Pharmacologic Classes and Agents**
- Inhibitors of Ergosterol Synthesis
  - *Ketoconazole*
  - *Itraconazole*
  - *Fluconazole*
  - *Voriconazole*
- Ergosterol-Binding Compounds
  - *Amphotericin B*
  - *Nystatin*
- Inhibitors of Thymidylate Synthase
  - *Flucytosine*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Fungi are free-living microorganisms that exist as **yeasts** (single-cell, round fungi), **molds** (filamentous fungi), or a combination of the two (so-called dimorphic fungi). All fungi are **eukaryotic** organisms. Because of their phylogenetic similarity, fungi and humans have homologous metabolic pathways for energy production, protein synthesis, and cell division. Consequently, *there is greater difficulty in developing selective antifungal agents than in developing selective antibacterial agents.* The success of many antibacterial agents has resulted from the identification of unique molecular targets in bacteria, emphasizing the necessity for identifying unique fungal targets that can be exploited.

Certain patient populations are particularly susceptible to fungal infections (mycoses). These populations include surgical and ICU patients, patients with prostheses, and immunocompromised patients. Immunocompromised patients, in particular, largely account for the rapid increase in the incidence of fungal infection over the past two decades; factors contributing to this trend include the extended use of broad-spectrum antibiotics, the increasing use of long-term intravenous catheters, and infection with the human immunodeficiency virus (HIV). In addition, the increasing successes of organ transplantation, immunosuppressive therapy, and cancer chemotherapy have contributed to the increasing number of patients who are chronically immunosuppressed.

Traditionally, the diagnosis of fungal infections has relied on culture-based methods and direct examination of specimens under light microscopy. However, the indolent growth of fungi makes culturing inefficient, and direct microscopic examination may not always be reliable, and cannot provide definitive speciation. These disadvantages have important clinical implications, because prognosis often correlates inversely with the duration of time from clinical presentation to accurate diagnosis. Consequently, one major focus of modern mycology is the development of rapid, nonculture-based methods of early diagnosis. New diagnostic techniques rely on polymerase chain reaction (PCR), western blot, antigen detection, and identification of fungal metabolites. Because these techniques are still considered investigational, however, they must be performed in parallel with the traditional culture-based methods.

Once a diagnosis is made, the treatment options for opportunistic fungal infections are limited, because: (1) only a small number of unique biochemical targets have been found; and (2) current mainstay treatments are ill-suited for the prolonged use necessary to eradicate systemic fungal infections. This chapter describes the existing pharmacologic agents and their molecular mechanisms of action. Most of these agents focus on one unique target, the fungal membrane. Two antifungal agents with clinical utility are also discussed in other chapters; flucytosine is included in Chapter 31, Pharmacology of Nucleotide Synthesis, while griseofulvin is included in Chapter 32, Pharmacology of DNA Replication and Mitosis.

## ■ Case

James Fuller, a 31-year-old HIV-positive male, presents to his physician with a 3-week history of fever, cough, and chest pain after touring southern California. His history is notable for past intravenous drug use. Clinical evaluation and chest x-ray reveal a left lower lobe infiltrate and left paratracheal adenopathy. Sputum cultures are positive for *Coccidioides immitis,* and blood tests are notable for an elevated titer of antibodies directed against this fungal pathogen. The physician makes a preliminary diagnosis of pulmonary coccidioidomycosis and prescribes a course of amphotericin B.

Over the next several days, however, Mr. Fuller does not improve. He goes to the emergency room with fever, chills, sweats, cough, fatigue, and headaches. His temperature is 100°F, but he shows no evidence of meningitis or peripheral adenopathy. Lung examination reveals diffuse wheezing over the left lung fields, noted on both inspiration and expiration. Bronchoscopy shows narrowing of the tracheal lumen by numerous mucosal granulomas from the left main-stem bronchus to the level of the midtrachea. Fungal culture grows *Coccidioides immitis;* a definitive diagnosis of chronic pulmonary coccidioidomycosis is made; the granulomas are bronchoscopically removed; and amphotericin B is continued. A week later, Mr. Fuller's symptoms begin to subside, amphotericin B is discontinued, and a course of fluconazole is prescribed.

## QUESTIONS

- **1.** What factors predisposed Mr. Fuller to fungal infection?
- **2.** What are the mechanisms of action of amphotericin B and fluconazole?
- **3.** What are the side effects that Mr. Fuller could experience as a consequence of treatment with amphotericin B and fluconazole?

## BIOCHEMISTRY OF FUNGAL MEMBRANES AND CELL WALLS

A unique biochemical target in fungi and a major target of antifungal drug therapy is the plasma membrane component **ergosterol.** This **sterol** differs structurally from cholesterol, which is the predominant sterol found in mammalian cell membranes. Membrane sterols are important for the maintenance of membrane structure and function. One of the steps in the synthesis of ergosterol involves the demethylation of **lanosterol,** a reaction catalyzed by the fungus-specific cytochrome P450 enzyme **14α-sterol demethylase.** All of the enzymes in the ergosterol synthesis pathway are compartmentalized in fungal **microsomes,** which contain an electron transport system identical to that found in mammalian liver microsomes. Inhibition of ergosterol synthesis compromises fungal membrane integrity and leads to fungal cell death. The 14α-sterol demethylase enzyme is the target of antifungal **azole** drugs (see below).

A second important target for antifungal therapy is the fungal **cell wall.** The cell wall is composed chiefly of **chitin,** but also contains **glucans, mannans,** and varying amounts of other structural proteins. Compounds that selectively target structural components of the fungal cell wall have recently been approved for clinical use (see below).

A third target is fungal adhesion. Adhesion to host cells is mediated by binding of a fungal **adhesin** to a host cell receptor. In yeasts, for example, aspartyl proteases and phospholipases mediate adhesion. Compounds are currently under development to antagonize adhesive interactions between fungal cells and mammalian cells.

## PATHOPHYSIOLOGY OF FUNGAL INFECTIONS

The pathogenesis of fungal infections is only partially understood. The first steps are adhesion and local colonization, typically on oral, vaginal, or gastrointestinal mucosal surfaces, although colonization of skin, respiratory epithelium, and foreign bodies (i.e., prosthetic devices) can also occur. Successful pathogens are subsequently able to invade the colonized surface and proliferate in deep tissue, sometimes reaching the systemic circulation. This step, which likely involves the action of lytic enzymes (analogous to those characterized in some bacterial species), is greatly accelerated by local tissue injury, such as that caused by cancer chemotherapy, ischemia, or the presence of a prosthetic device.

Immunocompetent hosts can generally prevent fungal pathogens from initiating infection. However, in patients who are HIV-positive or otherwise immunocompromised, fungal pathogens can become major contributors to illness. In decreasing order of significance, important lines of host defense include polymorphonuclear leukocytes, cell-mediated immunity, and humoral immunity.

Clinically, the lungs are the most common portal of entry for fungal infections. Mechanisms of **innate immunity,** involving the activation of **alveolar macrophages,** are responsible for clearing the initial infectious insult. If alveolar macrophages are unable to clear the inhaled fungus, then symptomatic fungal infections (mycoses) can develop. These mycoses vary greatly in their manifestations, but tend to be subacute to chronic with indolent, relapsing features. Acute disease is uncommon in fungal infections, because of the slow rate of fungal replication. Infectious mycoses can appear as superficial, cutaneous, subcutaneous, or systemic diseases. The treatment depends on the extent and tissue location of the infection.

## PHARMACOLOGIC CLASSES AND AGENTS

The ideal antifungal drug would have four characteristics. First, because diagnosing the fungal species involved in a given infection is difficult, the antifungal agent should be active against a broad spectrum of organisms. Second, drug toxicity should be low, allowing the extended dosing regimens required to eradicate these slow-growing organisms. Third, the drug should be available in multiple dosage forms (e.g., oral and intravenous preparations) to enable optimal management of infections based on their severity. Fourth, the drug should have good penetration into the cerebrospinal fluid (CSF), urine, and bone. Unfortunately, no available antifungal agent possesses more than two of these characteristics. The existing agents can be divided into drugs that inhibit ergosterol synthesis, drugs that disrupt fungal membranes by binding to ergosterol, and drugs that inhibit DNA synthesis (Fig. 36-1).

## INHIBITORS OF ERGOSTEROL SYNTHESIS

For decades, **amphotericin B** (see below) provided the only effective treatment for systemic mycoses. Although amphotericin B possesses potent antifungal activity, its use is often limited by significant toxicity. Because of limitations in the systemic use of amphotericin B, there was strong motivation to develop additional new drugs. This drug development program has led to the introduction of **azoles** to treat systemic mycoses. Representatives of this class include **ketoconazole, itraconazole, fluconazole, and voriconazole.** These drugs have now become the standard clinical therapies for systemic mycoses. Relative to amphotericin B, the azoles demonstrate more favorable adverse effect profiles, comparable clinical benefit, and more convenient dosing regimens. Pharmacokinetic and pharmacodynamic characteristics of each drug, including half-life, spectrum of action, and penetration into CSF, determine the clinical appropriateness of each agent for a given infection.

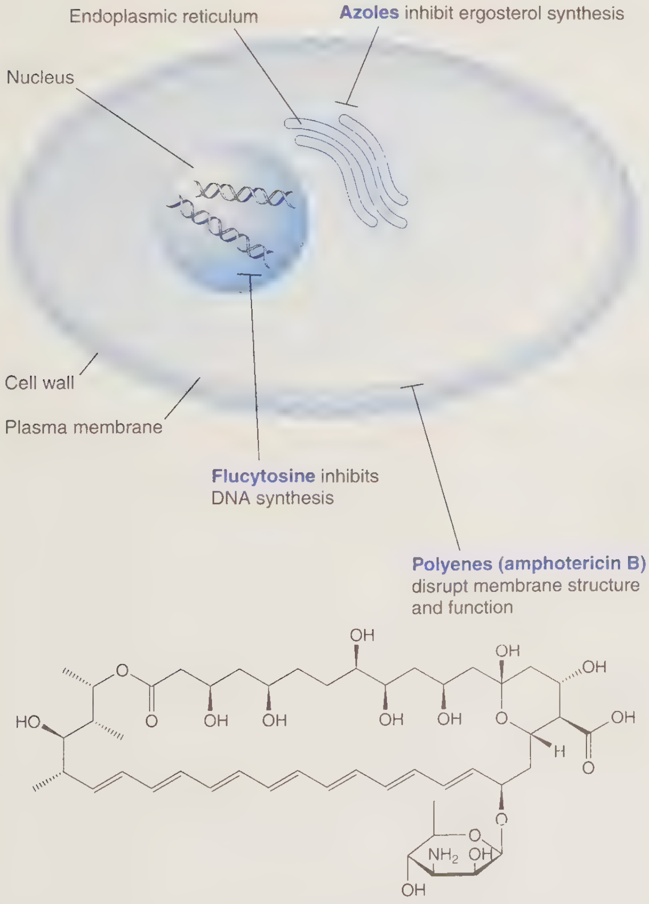

Endoplasmic reticulum
Azoles inhibit ergosterol synthesis
Nucleus
Cell wall
Plasma membrane
Flucytosine inhibits DNA synthesis
Polyenes (amphotericin B) disrupt membrane structure and function
OH
OH
OH
HO
O
O
OH
OH
OH
OH
O
OH
H
O
O
O
NH₂
OH
OH
Amphotericin B

**Figure 36-1. Cellular Targets of Antifungal Agents.** Currently available antifungal agents target three cellular components of fungi. Fungi synthesize ergosterol, a membrane sterol not present in mammalian cells. Azoles inhibit the synthesis of ergosterol in the endoplasmic reticulum of the fungal cell. Polyenes such as amphotericin B bind to ergosterol in the fungal membrane, causing disruption of membrane structure and function. Flucytosine is converted within the fungal cell to 5-fluorouracil, which inhibits DNA synthesis.

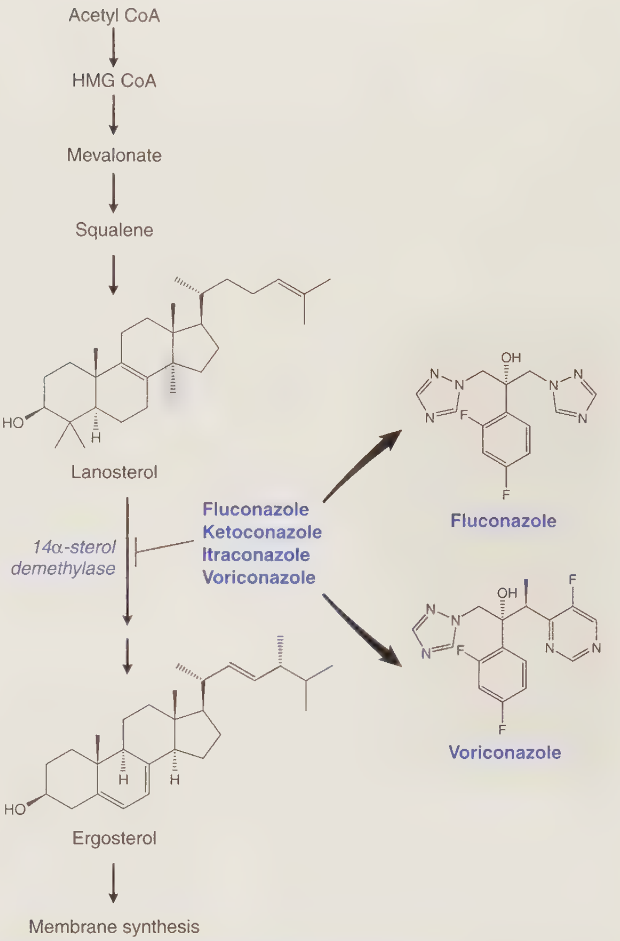

Acetyl CoA
HMG CoA
Mevalonate
Squalene
HO
H
Lanosterol
14α-sterol demethylase
Fluconazole
Ketoconazole
Itraconazole
Voriconazole
OH
N
N
N
N
N
N
F
F
Fluconazole
OH
F
N
N
N
N
N
F
F
Voriconazole
HO
H
H
Ergosterol
Membrane synthesis

**Figure 36-2. Mechanism of Action of Azoles.** Ergosterol is synthesized within fungal cells from acetyl CoA building blocks. (Note the similarity between this pathway and the biosynthetic pathway leading to cholesterol synthesis in mammalian cells, depicted in Fig. 22-7.) Azoles act by inhibiting the cytochrome P450 enzyme 14α-sterol demethylase, an enzyme not expressed in mammalian cells. Inhibition of this enzyme prevents the synthesis of ergosterol, which is the principal sterol in fungal membranes.

All azoles possess a similar mechanism of action: they inhibit the fungal cytochrome P450 14α-sterol demethylase, the enzyme responsible for converting lanosterol to ergosterol (Fig. 36-2). The resulting decrease in ergosterol synthesis leads to destabilization of fungal membranes and cell death. Azoles are not entirely selective for the fungal enzyme, in that they cause some inhibition of hepatic cytochrome P450 enzymes as well. The extent of hepatic P450 inhibition varies among the azoles; ketoconazole is the most potent, followed by itraconazole, voriconazole, and fluconazole. This inhibition of hepatic P450 can be exploited therapeutically. Cyclosporine, for example, is an immunosuppressant drug used in allogeneic kidney, liver, and heart transplant recipients to prevent graft rejection (see Chapter 43, Pharmacology of Immunosuppression). It is metabolized by hepatic cytochrome P450 enzymes and excreted in the bile. Cyclosporine can also be nephrotoxic and hepatotoxic. To minimize the risk of these toxicities, smaller doses of cyclosporine should be coadministered with a P450-inhibiting azole. Azoles also antagonize steroidogenesis in the adrenal gland and gonads, by inhibiting the P450-dependent enzymes 17,20-lyase and side-chain cleavage enzyme, both of which are necessary for steroid hormone synthesis (see Chapter 26, Pharmacology of the Adrenal Cortex). At therapeutic doses, only ketoconazole has shown clinically significant adrenal inhibition. Ketoconazole can, therefore, be used to inhibit androgen production in patients with advanced prostate cancer and to inhibit corticosteroid synthesis in patients with advanced adrenal cancer.

### Ketoconazole

Ketoconazole is available only as an oral agent. Its spectrum of action includes *Coccidioides immitis, Cryptococcus neoformans, Candida* species, *Histoplasma capsulatum, Blastomyces dermatitidis,* and a variety of dermatophytes. Its pharmacokinetic profile and side effects limit its clinical utility, however. Absorption depends on the conversion of ketoconazole to a salt in the acidic environment of the stomach. Thus, the drug cannot be used if the patient has achlorhydria or is receiving bicarbonate, antacids, H2-blockers, or proton pump inhibitors (see Chapter 44, Integrative Inflammatory Pharmacology: Peptic Ulcer Disease). Ketoconazole has poor penetration into the CSF and urine, which limits its efficacy in central nervous system and urinary tract infections. Although the side effect profile of ketoconazole is more favorable than that of amphotericin B (see below), use of ketoconazole poses significant risks. In about 20% of patients, the drug causes nausea, vomiting, or anorexia, and hepatic dysfunction occurs in 1–2% of individuals. Persistent adrenal insufficiency has also been reported after ketoconazole therapy. Its use for systemic therapy has been largely replaced by the use of newer azoles.

### Itraconazole

The absorption of orally administered itraconazole, like that of ketoconazole, is maximized in an acidic gastric environment. Unpredictable rates of absorption, however, may require blood level monitoring to achieve desirable therapeutic levels. Unreliable oral bioavailability favors the use of intravenous administration. Itraconazole is oxidized in the liver to form the bioactive metabolite **hydroxy-itraconazole,** which is more than 90% bound to plasma protein. Hydroxy-itraconazole acts to inhibit fungal cytochrome P450. Its advantage over fluconazole and ketoconazole is that itraconazole shows increased activity against aspergillosis, blastomycosis, and histoplasmosis. Itraconazole is not well transported into CSF, urine, or saliva; however, it can be used in certain meningeal infections because high levels are achieved in the meninges. Hepatotoxicity is the major adverse effect.

### Fluconazole

Compared to the other azoles, fluconazole has several advantages that make it the treatment of choice for cryptococcal meningitis and systemic candidiasis. Fluconazole enters the CSF effectively and has a favorable toxicity profile. However, the spectrum of activity for fluconazole against other fungal pathogens is limited. Some clinical success can be achieved against coccidioidomycosis and blastomycosis but, for these infections, fluconazole is considered second-line therapy.

Fungal resistance to fluconazole develops readily; *Candida* species are the most notable pathogens to develop resistance. Mechanisms of resistance include mutation of fungal P450 enzymes and overexpression of multidrug efflux transporter proteins.

The bioavailability of oral fluconazole, unlike that of ketoconazole and itraconazole, is not influenced by gastric pH. Oral bioavailability is nearly 100%. Once absorbed, fluconazole diffuses freely into CSF, sputum, urine, and saliva. Fluconazole is currently the most widely used antifungal drug.

### Voriconazole

Voriconazole is available in both oral and parenteral forms and is the drug of choice in the treatment of invasive aspergillosis and other molds such as *Fusarium* and *Scedosporium.* Voriconazole is associated with a significant improvement in outcomes compared to amphotericin, particularly in difficult-to-treat cases, such as allogeneic bone marrow transplant recipients, patients with central nervous system infection, and patients with disseminated infection. Its spectrum of activity includes *Candida* species and a number of the newly emerging fungi. It is ineffective in the treatment of mucormycosis. Voriconazole is fungicidal against essentially all species of *Aspergillus.* It significantly inhibits hepatic cytochrome P450 enzymes, and smaller doses of cyclosporine or tacrolimus are used when these drugs are combined with voriconazole. The intravenous formulation should not be used in patients with renal failure because the cyclodextrin excipient accumulates and causes CNS toxicity. Hepatic toxicity is common, but can usually be managed by decreasing the dose. Unusual visual symptoms (photophobia and colored lights) can occur when the blood level is at its peak, usually lasting for 30–60 minutes.

## ERGOSTEROL-BINDING COMPOUNDS

### Amphotericin B

Amphotericin B, a polyene antibiotic, is an important drug for the treatment of systemic fungal infections. Both its therapeutic effect and its toxicity are related to its affinity for cell membrane sterols. Fortunately, *the affinity of amphotericin for ergosterol is 500 times greater than its affinity for cholesterol.* Binding of amphotericin B to fungal membrane sterols increases membrane permeability, likely through a **pore-forming** mechanism, resulting in leakage of essential cellular contents and death of the cell. The concentration of membrane-associated ergosterol in a given fungal species determines whether amphotericin is fungicidal or fungistatic. Resistance to amphotericin B, although less frequent than with other antifungal agents, is attributable to a decrease in the ergosterol content of fungal membranes.

Because amphotericin B is highly insoluble, it is supplied as a buffered deoxycholate colloidal suspension, which is poorly absorbed from the gastrointestinal tract and must be administered intravenously. Once in the bloodstream, more than 90% of the drug binds rapidly to tissue sites, while the remainder is bound to plasma protein. Penetration of amphotericin B into CSF is extremely poor. Hence, **intrathecal therapy** may be necessary for serious meningeal disease. The drug also diffuses poorly into vitreous humor and amniotic fluid.

The toxicity of amphotericin B, and of the polyenes in general, is the limiting factor in their clinical use. Toxic reactions associated with amphotericin B are divided into three groups—immediate systemic reactions, renal reactions, and hematologic reactions. Systemic reactions can include "**cytokine storm,**" in which amphotericin B elicits release of **tumor necrosis factor-alpha (TNF-$\alpha$)** and **IL-1.** In turn, TNF-$\alpha$ and IL-1 cause fever, chills, and hypotension within the first several hours after drug administration. These responses can usually be minimized by allowing the blood concentration of the drug to fall, by decreasing the rate of drug administration, or by pretreating with antipyretic agents (e.g., acetaminophen, NSAIDs, or hydrocortisone).

Renal toxicity of amphotericin B is a serious adverse event. The mechanism of renal toxicity is unknown, but may be related to amphotericin-mediated afferent arteriole vasoconstriction leading to renal ischemia. Renal toxicity is often the limiting factor in determining the extent of the therapeutic response. It may be necessary to discontinue therapy temporarily if the blood urea nitrogen (BUN) exceeds 50 mg/dL or if the serum creatinine exceeds 3 mg/dL. (BUN and creatinine are surrogate measures of renal function.) Renal tubular acidosis, cylindruria (the presence of renal cell casts in the urine), and hypokalemia can occur to the extent that electrolyte replacement is required. In the introductory case, treatment with amphotericin was discontinued as soon as Mr. Fuller's acute symptoms had resolved, to prevent renal toxicity.

Hematologic toxicity of amphotericin B is also common; anemia is probably secondary to decreased production of erythropoietin.

The renal and hematologic toxicities of amphotericin are cumulative and dose-related. Attempts to minimize these toxicities include avoidance of other nephrotoxic drugs, such as aminoglycosides and cyclosporine, and maintenance of euvolemia to provide adequate renal perfusion. Despite these interventions, significant toxicity is often limiting, and may require stopping the administration of amphotericin B.

Attempts to reduce nephrotoxicity have led to the development of **lipid formulations** of amphotericin B. The strategy is to package amphotericin B in liposomes or other lipid carriers, with the goal of preventing high drug exposure to the proximal tubule of the nephron. **Amphotec, abelcet,** and **ambisome** are all FDA-approved lipid-containing preparations of amphotericin B. They are equal in efficacy to each other and to native amphotericin deoxycholate. They are less toxic than the native compound, but more expensive.

### Nystatin

A structural relative of amphotericin B, **nystatin** is a polyene antifungal that also acts by binding ergosterol and causing pore formation in fungal cell membranes. Because of severe systemic toxicity, the use of nystatin is strictly limited to topical infections of the skin and oral mucosa.

## INHIBITORS OF THYMIDYLATE SYNTHASE

### Flucytosine

Flucytosine is typically used in combination with amphotericin B to treat systemic mycoses because, when the drug is used as a single agent, resistance emerges rapidly. Although flucytosine has no inherent activity against *Aspergillus,* synergistic killing of *Aspergillus* can be demonstrated experimentally using flucytosine and amphotericin B. The spectrum of activity of flucytosine as a single agent is limited to candidiasis, cryptococcosis, and chromomycosis. The pharmacokinetic advantage of this drug is its large volume of distribution, with excellent penetration into the CNS, eyes, and urinary tract.

Flucytosine uptake by fungal cells depends on **cytosine-specific permeases** found only in fungal membranes. Thus, mammalian cells, which lack this transporter, are protected. Inside the cell, flucytosine is converted to 5-fluorouracil (5-FU), an antimetabolite used in cancer chemotherapy (see Chapter 31), by the enzyme **cytosine deaminase.** Subsequent reactions convert 5-FU to 5-fluorodeoxyuridylic acid (5-FdUMP), an inhibitor of DNA synthesis and cell division (Fig. 36-3). Mutations in the permeases or cytosine deaminase account for the rapid development of resistance when the drug is used as a single agent. Although mammalian cells lack the cytosine-specific permeases and cytosine deaminase, fungi and bacteria in the intestine convert flucytosine into 5-fluorouracil, which is toxic to human cells. Dose-dependent toxicity includes bone marrow suppression, nausea, vomiting, diarrhea, and hepatic dysfunction. Flucytosine is contraindicated during pregnancy.

## *Conclusion and Future Directions*

The biochemical similarities between fungal cells and human cells limit the efficacy of many antifungal drugs. Only a small number of unique fungal targets have been identified (e.g., ergosterol, cytosine permease). The major drugs currently available—azoles, amphotericin B, and flucytosine—have narrow therapeutic indices.

Current developments in antifungal pharmacology include azoles with more favorable pharmacokinetic properties, and inhibitors of cell wall synthesis. Several new azoles, including **ravuconazole** and **posaconazole,** may have broader clinical utility than the currently available azoles. Inhibitors of glucan synthesis, while limited in their spectrum of action because of fungal variations in glucase expression, may provide a new class of antifungal agents. For example, **echinocandins**—cyclic hexapeptides that inhibit the biosynthesis of beta-glucans—have recently been shown to be effective agents in the treatment of invasive candidiasis and candidemia. These compounds lack the more severe toxic side effects of the polyene amphotericin B and are more potent than the azole fluconazole against nonalbicans species. **Caspofungin** is the first compound in this class to be approved. In addition to their anticandidal activity, these cell wall synthesis inhibitors have antiaspergillus activity. Because of their different mechanisms of action, it should be possible for these compounds to be used in combination with azoles or polyenes (similar to combination antibacterial therapies). For example, an agent that inhibits fungal cell wall synthesis could potentiate the action of a second drug that requires access to fungal microsomes to be effective.

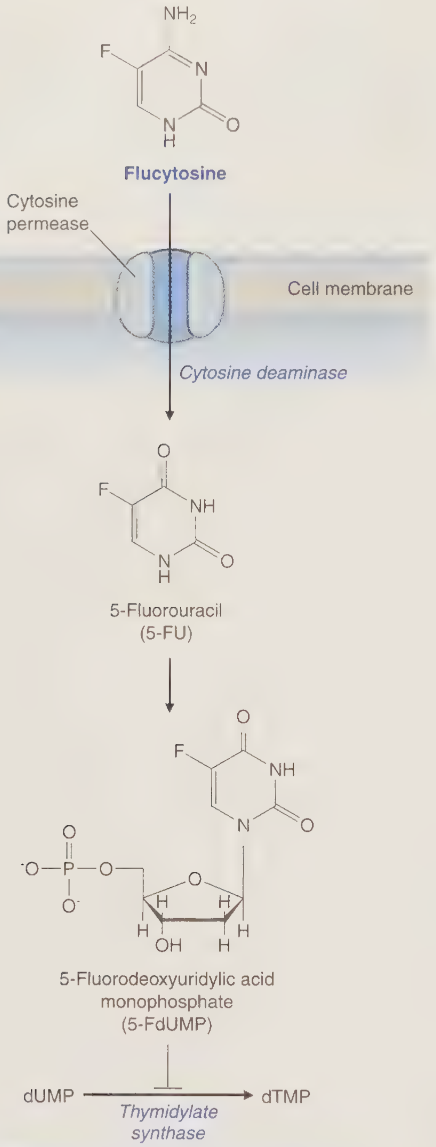


**Figure 36-3. Mechanism of Action of Flucytosine.** Flucytosine enters the fungal cell via a transmembrane cytosine permease. Inside the cell, cytosine deaminase converts flucytosine to 5-fluorouracil (5-FU), which is subsequently converted to 5-fluorodeoxyuridylic acid monophosphate (5-FdUMP). 5-FdUMP inhibits thymidylate synthase and, thereby, blocks the conversion of deoxyuridylate (dUMP) to deoxythymidylate (dTMP). In the absence of dTMP, DNA synthesis is inhibited.

## *Suggested Readings*

Graybill JR. The future of antifungal therapy. Clin Infect Dis. 1996; 22:S166–178. (*Describes basic principles of antifungal therapy and how the pharmacology will evolve in the future.*)

Ryan KJ. Characteristics of fungi. In: Sherris JC, ed. Medical Microbiology: An Introduction to Infectious Diseases. 3rd ed. Norwalk, CT: Appleton & Lange; 1994. (*Good summary of fungal growth, classification, and laboratory diagnosis.*)

Ryan KJ. Pathogenesis, immunity, and chemotherapy of fungal infections. In: Sherris JC, ed. Medical Microbiology: An Introduction to Infectious Diseases. 3rd ed. Norwalk, CT: Appleton & Lange; 1994. (*Good outline of the pathogenesis of fungal mycoses and immune defense mechanisms.*)

Sarosi GA, Davies SF. Fungal Diseases of the Lung. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2000. (*Discussion of general mycology and clinical pathophysiology.*)

Wiederhold NP, Lewis RE. The echinocandin antifungals: an overview of the pharmacology, spectrum and clinical efficacy. Expert Opin Investig Drugs. 2003;12:1313–1333. (*Basic overview of this new class of antifungal agents.*)

## Drug Summary Table Pharmacology of Fungal Infections

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF ERGOSTEROL SYNTHESIS**<br>*Mechanism—Inhibit 14α-sterol demethylase, the enzyme that initiates the conversion of lanosterol to ergosterol* | | | | |
| **Fluconazole**<br>**Itraconazole**<br>**Ketoconazole**<br>**Voriconazole** | *Candida, Cryptococcus, Histoplasma, Blastomyces, Coccidioides, Aspergillus* | Nausea, vomiting, abdominal pain, diarrhea<br>Hepatotoxicity (rare) | Inhibit CYP3A4 → ↓ metabolism of warfarin and cyclosporine | |
| **AGENTS THAT BIND TO ERGOSTEROL**<br>*Mechanism—Destabilize fungal cell membrane and increase cell membrane permeability* | | | | |
| **Amphotericin B** | Severe fungal infections | Fevers, chills<br>Nephrotoxicity<br>Anemia | Aminoglycosides and cyclosporine ↑ nephrotoxicity<br>May be antagonistic with azoles | Available as IV formulation, liposomal preparation, lipid complex<br>Liposomal preparation may ↓ nephrotoxicity |
| **Nystatin** | Topical fungal infections, usually oral candidiasis | Contact dermatitis<br>Stevens-Johnson syndrome | | Used as a mouthwash or vaginal insert only |
| **INHIBITORS OF THYMIDYLATE SYNTHASE**<br>*Mechanism—Fungal cytosine deaminase converts flucytosine to 5-fluorouracil, a potent inhibitor of thymidylate synthase* | | | | |
| **Flucytosine** | *Candida, Cryptococcus* | Nausea, vomiting, diarrhea<br>Hepatic dysfunction<br>Bone marrow suppression | Pregnancy | Used together with amphotericin B |

UNIVERSITY COLLEGE CORK Library

# 37

# Pharmacology of Parasitic Infections

Vinh Chung, Ehrin J. Armstrong, John Samuelson, and Edward T. Ryan

**Introduction**
**Malarial Plasmodia**
**Cases 1 & 2**
  Physiology of Malarial Plasmodia
    *Life Cycle*
    *Heme Metabolism*
    *Electron Transport Chain*
  Pharmacology of Anti-Malarial Agents
    *Inhibitors of Heme Metabolism*
      *Chloroquine*
      *Quinine and Quinidine*
      *Mefloquine*
      *Artemisinin*
    *Inhibitors of the Electron Transport Chain*
      *Primaquine*
      *Atovaquone*
**Other Protozoa**
**Case 3**
  Physiology of Luminal Protozoa
    *Life Cycle of Entamoeba histolytica*
    *Fermentation Pathways*
  Pharmacology of Anti-Protozoal Agents
    *Metronidazole*
    *Other Anti-Protozoal Agents*
**Helminths**
**Case 4**
  Physiology of Helminths
    *Life Cycle of Onchocerca volvulus*
    *Neuromuscular Activity*
  Pharmacology of Anti-Helminthic Agents
    *Agents That Interrupt Neuromuscular Activity*
      *Ivermectin*
      *Piperazine*
      *Pyrantel Pamoate*
    *Other Anti-Helminthic Agents*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

More than one billion people worldwide are infected with parasites. Parasites of medical importance include protozoa (such as the organisms that cause malaria, toxoplasmosis, giardiasis, amebiasis, leishmaniasis, and trypanosomiasis) and helminths ("worms"). Worms that infect humans include cestodes ("flat worms" or "tape worms," such as the worm that causes taeniasis), nematodes ("round worms," such as the worms that cause filariasis, strongyloidiasis, and ascariasis), and trematodes ("flukes," such as the worm that causes schistosomiasis).

Ideally, antiparasitic drugs should be targeted to structures or biochemical pathways present or accessible only in parasites. Many antiparasitic drugs act by unknown or poorly defined mechanisms of action, however. This chapter focuses on a number of the better defined agents, including those active against *Plasmodia* spp. (which cause malaria), *Entamoeba histolytica* (which causes amebiasis), and *Onchocerca volvulus* (which causes onchocerciasis, a filarial infection referred to as "river blindness"). In each of these cases, antiparasitic agents interfere with metabolic requirements of the parasite: the dependence of malarial plasmodia on heme metabolism, the dependence of luminal parasites on specific fermentation pathways, and the dependence of helminths on neuromuscular activity. These three examples are not all-inclusive, but rather emphasize opportunities to use or design pharmacologic agents to interrupt metabolic requirements specific to parasites.

## MALARIAL PLASMODIA

Each year, 300–500 million individuals in more than 90 countries develop malaria, and 1.5–2.7 million individuals die of malaria. Malaria is the most important parasitic disease of humans, and one of the most important infections of humans. Human malaria is caused by one of four species of plasmodial protozoal parasites: ***Plasmodium falciparum, P. vivax, P. malariae,*** and ***P. ovale.*** The most serious type of malaria is that caused by *P. falciparum.*

## Case 1

Binata, a 3-year-old girl living in Senegal, is in good health when, one day, she begins to feel hot, has sweats and shaking chills, stops eating, and becomes intermittently listless and lethargic. Several days later, these symptoms climax in a seizure and coma, prompting Binata's parents to rush her to the local health care clinic. In the clinic, the unconscious child's neck is supple, but she is febrile to 103°F. Her lungs are clear to auscultation, and there is no rash. A smear of Binata's peripheral blood discloses *P. falciparum* ring trophozoites in approximately 10% of her erythrocytes. Binata is given the only antimalarial medicines available at the clinic, chloroquine and pyrimethamine-sulfadoxine; however, the child does not improve, and she dies within 24 hours of being brought to the clinic.

### QUESTIONS

1. Why did Binata die?
2. Why did Binata not improve after receiving antimalarial drugs?
3. How often does a child die of malaria?

## Case 2

Mr. Gupta is a 36-year-old married software engineer who was born and raised in India. He comes to the United States and is completely well for 6 months. He then begins to experience episodes of fever, headache, and body aches. Three months later, he goes to his physician, who diagnoses malaria and prescribes chloroquine for treatment. Therapy with chloroquine resolves his symptoms completely. However, Mr. Gupta notes recurrence of fevers and the other symptoms 3 months later, and returns to his doctor's office.

### QUESTIONS

1. What is a likely explanation for the return of Mr. Gupta's fever?
2. How can Mr. Gupta's treatment be modified so that his illness will not return?

## PHYSIOLOGY OF MALARIAL PLASMODIA

### Life Cycle

The life cycle of malaria involves a parasite, a mosquito vector, and a human host (*Fig. 37-1*). An ***Anopheles*** spp. mosquito can ingest sexual forms of malarial parasites (gametocytes) when taking a blood meal from an infected human. After fusion of the male and female gametocytes and maturation of the zygote within the mosquito, **sporozoites** are released from an oocyst. The sporozoites, which migrate to the mosquito's salivary glands, can be inoculated into the blood of another human host during a subsequent blood meal. In the human, sporozoites leave the blood and multiply in the liver, forming **tissue schizonts.** This *exo-erythrocytic hepatic stage* is asymptomatic. In a typical *P. falciparum* infection, 1 to 12 weeks after the infective bite, the liver cells release parasites into the bloodstream as **merozoites.** A single sporozoite can produce over 30,000 merozoites. Merozoites invade erythrocytes, multiply asexually, and form **blood schizonts.** This is the *erythrocytic stage. Infected erythrocytes eventually rupture, releasing another generation of merozoites that continue the erythrocytic cycle. Rare merozoites also mature into gametocytes. Ingestion of these circulating gametocytes by an appropriate mosquito completes the life cycle.* The clinical symptoms of malaria, most distinctively fever, are caused by the intravascular lysis of erythrocytes and subsequent release of merozoites into the blood. The fevers that Binata and Mr. Gupta experienced were associated with these hemolytic episodes.

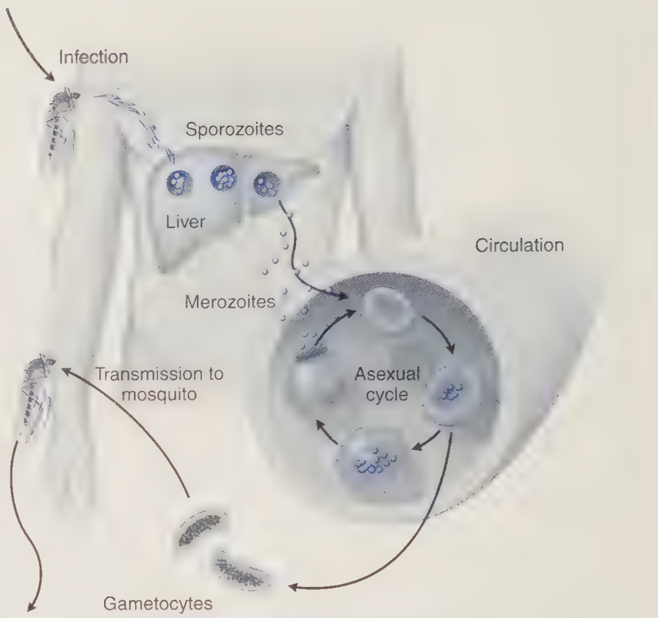


**Figure 37-1. Life Cycle of Malaria.** Malarial plasmodia have a complex life cycle that relies on both humans and *Anopheles* spp. mosquitoes. Gametocytes from an infected human are transferred to a mosquito during a blood meal. In the mosquito stomach, a zygote forms and matures to become an oocyst on the outside wall of the stomach (not shown). Sporozoites released from the oocyst migrate to the salivary glands. During its next blood meal, the mosquito transfers *Plasmodium* spp. sporozoites from its saliva to another human. Sporozoites enter the host's bloodstream and travel to the liver. Sporozoites replicate in the liver and then lyse infected hepatocytes, releasing merozoites into the circulation. Merozoites infect erythrocytes, undergoing asexual cycles of erythrocytic infection and lysis. Some merozoites differentiate into gametocytes, which can be ingested by another mosquito and, thereby, continue the cycle of infection. *P. vivax* and *P. ovale* can also form dormant hypnozoites, which can remain in infected hepatocytes for months to years before release into the circulation (not shown).

Binata unfortunately developed cerebral malaria due to *P. falciparum. P. falciparum*-infected erythrocytes express "knobs" on their surface that are composed of both host and parasite proteins. Parasite proteins include PfEMP-1, a protein family comprised of approximately 100–150 gene products that mediate attachment of infected erythrocytes to cellular receptors—including CD36, ICAM-1, ELAM-1, and chondroitin sulfate—on endothelial surfaces in the human host. Such endothelial attachment lessens the amount of time for which infected erythrocytes circulate systemically, thereby decreasing the likelihood that infected erythrocytes will be cleared via splenic sequestration.

This intravascular binding during a malarial episode occurs only during *P. falciparum* infection and contributes to intravascular "sludging" of erythrocytes. Such sludging accounts, in large part, for the pathophysiology of malaria caused by *P. falciparum.* Sludging can affect any organ, including the brain, lungs, and kidneys; damage to these organs leads to tissue hypoxia, focal necrosis, and hemorrhage. In Binata's case, the brain was involved (so-called "cerebral malaria"). Untreated, cerebral malaria is almost uniformly fatal, and even with optimal treatment, cerebral malaria has a case fatality rate exceeding 20%.

Binata was treated with two drugs that have historically been quite important in treating individuals with malaria, but which, unfortunately, are now ineffective in many places in the world because of widespread drug-resistant *P. falciparum.* Largely because of their low cost and availability, these drugs (chloroquine and a fixed combination of pyrimethamine and sulfadoxine) are still widely used in many developing areas of the world to treat older children and adults with partial immunity to malaria, but these agents have little clinical use in treating nonimmune individuals such as Binata. Unfortunately, Binata's story is all too common. On average, worldwide, a child dies of malaria every 20 seconds; of these deaths, more than 90% occur in sub-Saharan Africa, more than 90% occur in children under 5 years of age, and more than 95% are caused by *P. falciparum* infection. No pharmacologic agent has yet been developed that interferes with the recently elucidated role of PfEMP-1 in the endothelial attachment of malarially infected erythrocytes.

In Mr. Gupta's case, a peripheral blood smear showed *P. vivax* parasites inside his erythrocytes. Because *P. falciparum* and *P. malariae* infections involve only one cycle of hepatic cell invasion, drugs that eliminate these species from erythrocytes are usually sufficient to clear the infection. Unfortunately, *P. vivax* and *P. ovale* also have dormant hepatic forms (**hypnozoites**) that release merozoites over months to years. Individuals infected with *P. vivax* or *P. ovale,* therefore, should be treated with agents that are effective against not only blood-stage plasmodia, but also liver-stage parasites (see below). Because chloroquine does not eliminate hepatic forms of *P. vivax* and *P. ovale,* Mr. Gupta's *P. vivax* infection recurred.

## Heme Metabolism

Plasmodia have a limited capacity for de novo amino acid synthesis; instead, they rely on amino acids released from ingested host **hemoglobin** molecules. Within red blood cells, plasmodia degrade hemoglobin in a digestive vacuole, which is an elaborate lysosome with an acidic pH (Fig. 37-2). Hemoglobin is sequentially degraded to its constituent amino acids by plasmodial aspartic proteases (plasmepsins), a cysteine protease (falcipain), and a metalloprotease (falcilysin). Degradation of hemoglobin releases protonated basic amino acids and a toxic heme metabolite, ferriprotoporphyrin IX. Ferriprotoporphyrin IX is detoxified by polymerization to

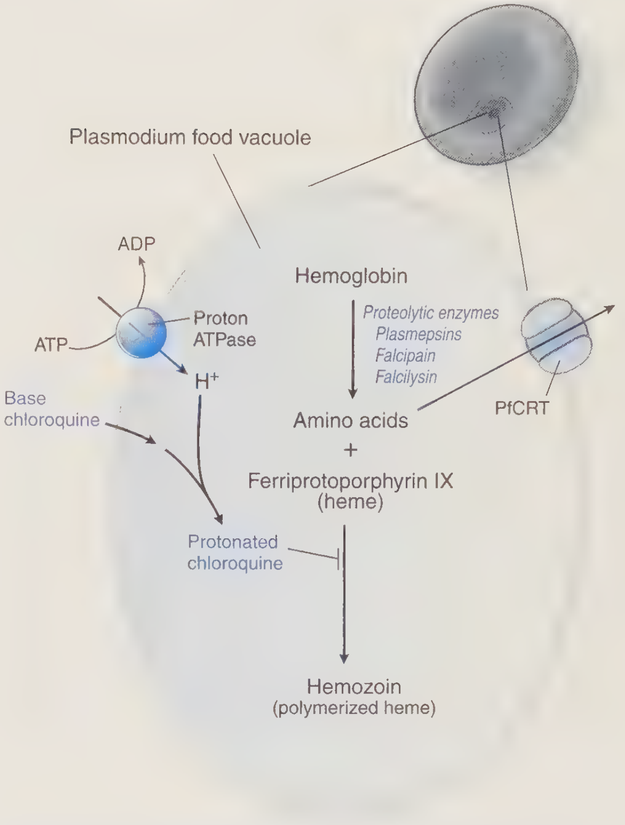


**Figure 37-2. Proposed Mechanisms of Heme Metabolism in the Plasmodial Food Vacuole.** Malarial plasmodia possess a specialized food vacuole that maintains an acidic intravacuolar environment by the action of a proton ATPase in the vacuolar membrane. Within the vacuole, human hemoglobin is used as a food source. Hemoglobin is proteolyzed to amino acids by several plasmodial-derived proteolytic enzymes, including plasmepsins, falcipain, and falcilysin. Protonated amino acids are then removed from the food vacuole through the PfCRT transporter. Degradation of hemoglobin also releases heme (ferriprotoporphyrin IX). Free ferriprotoporphyrin IX can react with oxygen to produce the superoxide anion ($O_2^-$); oxidant defense enzymes, which may include plasmodial-derived superoxide dismutase and catalase, convert the potentially cytotoxic superoxide to $H_2O$ (not shown). Plasmodia polymerize ferriprotoporphyrin IX into the nontoxic derivative hemozoin; evidence suggests that polymerization requires the activity of positively charged histidine-rich proteins (not shown). The iron moiety in ferriprotoporphyrin IX can also be oxidized from the ferrous ($Fe^{2+}$) to the ferric ($Fe^{3+}$) state, with concomitant production of hydrogen peroxide ($H_2O_2$). Many antimalarial agents are thought to disrupt the process of malarial heme metabolism; proposed mechanisms of drug action include inhibition of heme polymerization, enhancement of oxidant production, and reaction with heme to form cytotoxic metabolites. The inhibition of ferriprotoporphyrin IX polymerization by protonated hloroquine is shown.

crystalline hemozoin. If ferriprotoporphyrin IX does not polymerize, it causes lysosomal membrane damage and toxicity to the malarial parasites. Quinoline antimalarials (see below) are believed to act by inhibiting heme polymerization, thereby creating an environment that is toxic to intraerythrocytic plasmodia.

## Electron Transport Chain

Malarial plasmodia also possess mitochondria with a tiny genome (approximately 6 kB) that encodes only three **cytochromes** (large protein complexes involved in electron transport and oxidative phosphorylation). These cytochromes, together with a number of mitochondrial-targeted proteins derived from the plasmodial nuclear genome, encode a rudimentary electron transport chain similar in basic organization to that found in mammals (Fig. 37-3). In this electron transport chain, integral proteins of the mitochondrial inner membrane are reduced and then oxidized as they transport electrons from one intermediate protein to another. The energy liberated by electron transport is used to drive proton pumping across the mitochondrial membrane, and the energy gradient created by this proton pumping drives ATP synthesis. In this electron transport chain, oxygen is the final electron acceptor, resulting in the reduction of oxygen to water.

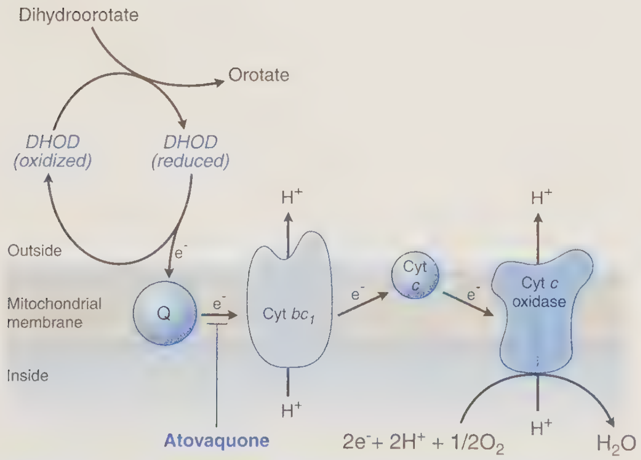


**Figure 37-3. The Mitochondrial Electron Transport Chain in Plasmodia.** The electron transport chain consists of a series of oxidation/reduction steps that culminate in the donation of electrons to oxygen, forming water. In plasmodia, the electron transport chain acts as an electron acceptor for reduced dihydroorotate dehydrogenase (DHOD), an enzyme that is essential for plasmodial pyrimidine synthesis. In this cascade, reduced ubiquinone (Q) transfers electrons to the cytochrome *bc*$_1$ complex (Cyt *bc*$_1$), which then passes electrons to cytochrome *c* (Cyt *c*) and, finally, to cytochrome *c* oxidase (Cyt *c* oxidase). In a 4-electron reduction of molecular oxygen (shown here as the half-reaction), cytochrome *c* oxidase donates electrons to oxygen to form water. This chain of electron transfers also involves the pumping of protons across the mitochondrial membrane by Cyt *bc*$_1$ and Cyt *c* oxidase; the resulting electrochemical gradient of protons is used later to generate ATP (not shown). Atovaquone antagonizes the interaction between ubiquinone and the plasmodial cytochrome *bc*$_1$ complex, thereby disrupting pyrimidine synthesis by preventing the regeneration of oxidized DHOD.

Plasmodia derive most of their ATP directly from glycolysis, and probably do not use mitochondrial electron transport as a significant source of energy. However, plasmodia do rely on electron transport for the oxidation of key enzymes involved in nucleotide synthesis. For example, **dihydroorotate dehydrogenase** (DHOD), the enzyme that mediates an early step in pyrimidine synthesis (see Chapter 31, Pharmacology of Nucleotide Synthesis), catalyzes the oxidation of dihydroorotate to orotate. As part of this reaction, DHOD is reduced, and the enzyme must be re-oxidized before it can continue with another cycle of catalysis. **Ubiquinone,** an integral membrane protein located near the beginning of the electron transport chain, accepts electrons from reduced DHOD, thus regenerating the oxidized form of DHOD necessary for pyrimidine synthesis. Because plasmodia depend on de novo pyrimidine synthesis for DNA replication, interrupting the ability of ubiquinone to oxidize DHOD can disrupt plasmodial DNA replication (see below).

# PHARMACOLOGY OF ANTI-MALARIAL AGENTS

The currently available antimalarial agents target four physiologic pathways in plasmodia—heme catabolism (chloroquine, quinine, mefloquine, and artemisinin), electron transport (primaquine and atovaquone), folate synthesis (sulfadoxine and pyrimethamine), and protein translation (clindamycin, doxycycline, and tetracycline). The following section discusses the pharmacologic agents that target heme catabolism and electron transport; inhibitors of folate synthesis and protein translation are discussed in Chapter 31, Pharmacology of Nucleotide Synthesis, and Chapter 33, Pharmacology of Transcription and Translation, respectively.

Clinically, antimalarials can be classified into agents used for prophylaxis (to prevent malaria in individuals residing in or traveling through a malaria zone), agents used for treating individuals with acute blood-stage malaria, and agents used to eliminate hypnozoite liver-stage malarial infections. Generally, agents used for prophylaxis must be well tolerated and easy to administer.

## Inhibitors of Heme Metabolism

Agents that disrupt intra-erythrocytic malarial parasites have, for centuries, been the foundation of antimalarial treatment regimens. Most of these compounds are congeners of quinoline and, as a result, are all believed to possess similar mechanisms of action. Artemisinin, discussed at the end of this section, is also thought to act by inhibiting heme metabolism, although its structure is different from that of the quinolines.

### *Chloroquine*

For the past two thousand years, humans have used the roots of *Dichroa febrifuga* or the leaves of hydrangea in the treatment of individuals with malaria. More recently, the bark of the **cinchona** tree was found to be a more effective remedy. In all these plants, a **quinoline** compound is the pharmacologically active antiplasmodial agent. **Chloroquine,** a 4-aminoquinoline, was introduced in 1934 for use in the treatment of malaria. Chloroquine is a weak base that, in its neutral form, freely diffuses across the membrane of the parasite's food vacuole. Once inside the acidic environment of the vacuole, chloroquine is rapidly protonated, making it unable to diffuse out of the vacuole. As a result, protonated chloroquine accumulates to high concentrations inside the parasite's food vacuole, where it binds to ferriprotoporphyrin IX and inhibits the polymerization of this heme metabolite. Accumulation of unpolymerized ferriprotoporphyrin IX leads to oxidative membrane damage and is toxic to the parasite. *Chloroquine thus poisons the parasite by preventing the detoxification of a toxic product of hemoglobin catabolism* (Fig. 37-2).

Chloroquine is concentrated by as much as 100 times in parasitized erythrocytes as compared to uninfected erythrocytes. In addition, the concentration of chloroquine required to alkalinize lysosomes of mammalian cells is much higher than that needed to raise the pH in malarial food vacuoles. Therefore, chloroquine is relatively nontoxic to humans, although the drug commonly causes pruritus in darkly pigmented individuals, and it can exacerbate psoriasis and porphyria. Taken in supratherapeutic doses, however, chloroquine can cause vomiting, retinopathy, hypotension, confusion, and death. In fact, chloroquine is used globally in many suicides each year (largely because it is inexpensive, available, and toxic at high doses), and accidental ingestion by children can be fatal.

When first introduced, chloroquine was a first-line drug used against all types of malaria; however, it is now ineffective against most strains of *P. falciparum* in Africa, Asia, and South America (Fig. 37-4). Hypotheses regarding the mechanisms responsible for chloroquine resistance are based on the finding that chloroquine-resistant plasmodia accumulate less chloroquine inside food vacuoles than chloroquine-sensitive plasmodia do. The strongest data suggest that altered drug efflux in the parasite's food vacuole best accounts for the resistance of *P. falciparum* to chloroquine. In the food vacuole, protonated amino acids are generated by the parasite as it degrades hemoglobin. These protonated amino acids exit the lysosome by means of a transmembrane protein called PfCRT, encoded by *pfcrt* on *P. falciparum* chromosome 7. A number of mutations in PfCRT have been associated with chloroquine resistance; a substitution of threonine for lysine at position 76 (K76T) is highly correlated with chloroquine resistance. This mutated PfCRT probably pumps protonated chloroquine out of the food vacuole. This altered pump action could also be detrimental to the parasite, perhaps because of altered amino acid export and/or changes in vacuole pH. Many *P. falciparum* strains with mutations in *pfcrt* carry a second mutation in the gene *pfmdr1* encoding Pgh1, a food vacuole membrane protein involved in pH regulation. It is speculated that this second mutation provides a "corrective" action that allows chloroquine-resistant *P. falciparum* to continue growth in the presence of a *pfcrt* mutation.

Chloroquine remains the drug of choice for treating most individuals with malaria caused by *P. vivax, P. ovale, P. malariae,* and chloroquine-sensitive strains of *P. falciparum.* It can also be used prophylactically to prevent malaria caused by sensitive strains of plasmodia.

### Quinine and Quinidine

**Quinine** is an alkaloid that consists of a quinoline ring linked by a secondary carbinol to a quinuclidine ring. Its optical isomer, **quinidine,** has identical pharmacologic actions. Because of quinine's structural similarity to other antimalarial quinolines, quinine is thought to attack plasmodia by the mechanism described above. Quinine has also been shown to intercalate into DNA through hydrogen bonds, thus inhibiting DNA strand separation, transcription, and translation. The overall effect is a decrease in the growth and replication of the erythrocytic form of plasmodia. Quinine and quinidine are used to treat individuals with acute blood-stage malaria,

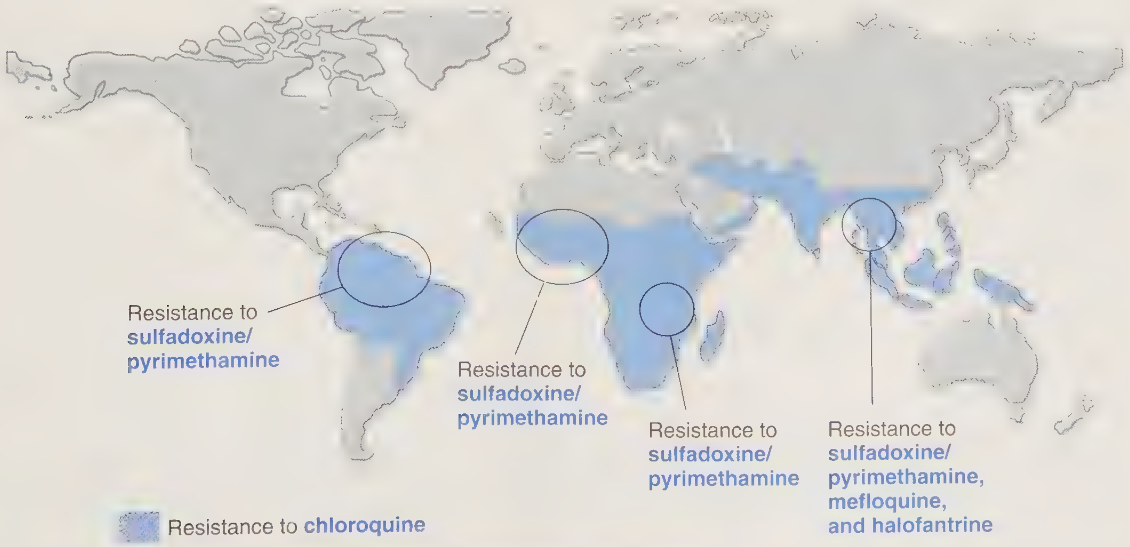

Resistance to sulfadoxine/ pyrimethamine
Resistance to sulfadoxine/ pyrimethamine
Resistance to sulfadoxine/ pyrimethamine
Resistance to sulfadoxine/ pyrimethamine, mefloquine, and halofantrine
▨ Resistance to chloroquine

Figure 37-4. Geographic Distribution of Drug-Resistant *Plasmodium falciparum.* Historically, chloroquine has been the drug of choice for prophylaxis and treatment of individuals with *P. falciparum* malaria. Unfortunately, *P. falciparum* is now resistant to chloroquine in most areas of the world (blue shading). In many areas, *P. falciparum* is also resistant to other antimalarial agents, including sulfadoxine–pyrimethamine, mefloquine, and halofantrine. (Halofantrine is associated with potentially lethal cardiac toxicity, and is therefore seldom used.)

and are not used prophylactically. Use of quinine can cause **cinchonism,** a syndrome that includes tinnitus, deafness, headaches, nausea, vomiting, and visual disturbances. Quinine and quinidine can also prolong the cardiac QT interval.

### Mefloquine

**Mefloquine** is a quinoline compound that is structurally related to many other antimalarial agents. Unlike quinine, mefloquine does not bind to DNA. Its exact mechanism of action is unknown, although mefloquine seems to disrupt clumping of hemozoin inside intra-erythrocytic malarial parasites. Mefloquine has a number of side effects including nausea, cardiac conduction abnormalities (including bradycardia, prolongation of the QT interval, and arrhythmia), and neuropsychiatric side effects including vivid dreams/nightmares, insomnia, anxiety, depression, hallucinations, seizures and, rarely, psychosis. The mechanism(s) responsible for these side effects is unknown. Mefloquine can be used both therapeutically and prophylactically.

### Artemisinin

**Artemisinin** has been used in China (where it is known as *qinghao*) for centuries in the treatment of individuals with fever. The compound is a cyclic endoperoxide that, when activated by free or heme-bound iron, forms a carbon-centered free-radical compound (Fig. 37-5). This free radical has the ability to alkylate many proteins, and it can alkylate heme as well. The specificity of the drug for plasmodial-infected erythrocytes is unknown—two potential sources of specificity include artemisinin's requirement for heme for free radical formation, and artemisinin's preferential accumulation in plasmodia.

Artemisinin is generally well tolerated, but studies have suggested that artemisinin and its derivatives can have neurotoxic and cardiotoxic side effects. In laboratory animals, artemisinin has been shown to cause brainstem neuropathy; this potentially lethal side effect has not been observed in humans. High doses of artemisinin can also prolong the QT interval, placing certain patient populations at risk for cardiac arrhythmias.

Currently, artemisinin is used in many parts of Africa and Southeast Asia as a first-line treatment for individuals with acute malaria infection. There have not been any reported clinical cases of resistance to artemisinin, but laboratory studies with plasmodia suggest that resistance is possible. Because of concern over the development of resistance and the short half-life (from 1–11 hours) of artemisinin derivatives, artemisinin is usually coadministered with a second agent. Artemisinin is not effective as a prophylactic agent against malaria.

## Inhibitors of the Electron Transport Chain

Although the electron transport chain is a ubiquitous feature of eukaryotic cells, two agents have been developed that seem to interrupt the plasmodial electron transport chain selectively. This selectivity is due to different molecular structures of the same biochemical target, rather than the presence of a unique enzymatic pathway in plasmodia (see Chapter 30, Principles of Antimicrobial and Antineoplastic Pharmacology).

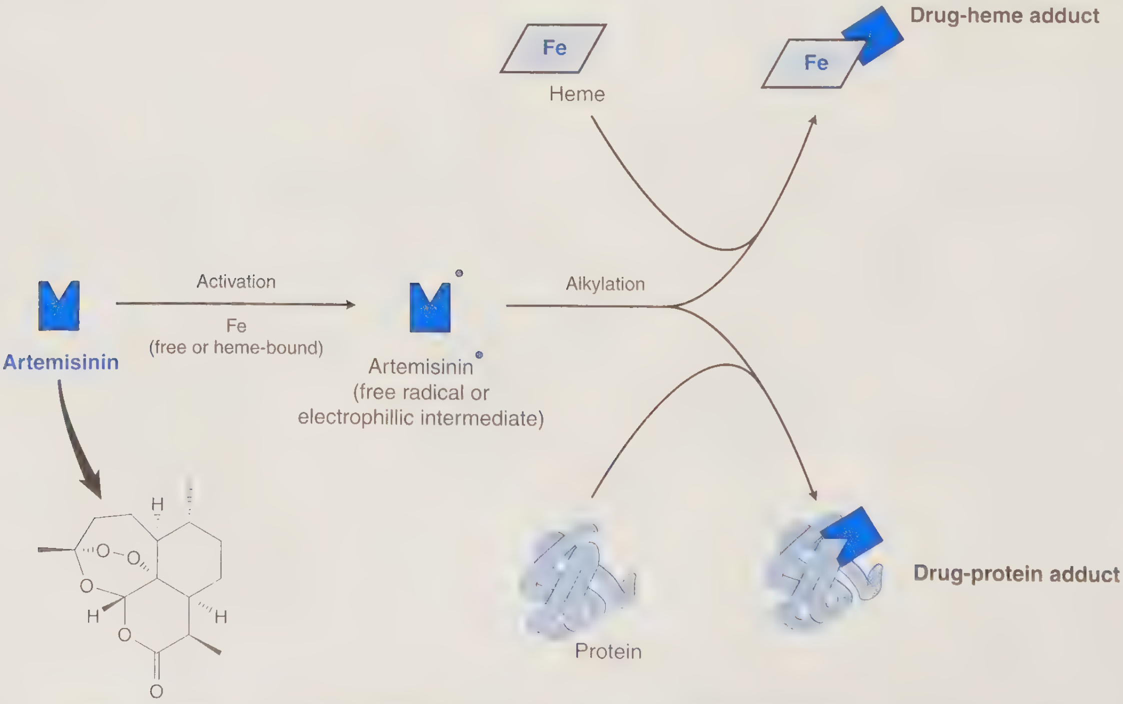


**Figure 37-5. Proposed Mechanism of Action of Artemisinin.** Artemisinin is a cyclic endoperoxide compound that forms a free radical after activation by iron (Fe). This free radical is able to alkylate such macromolecules as heme and proteins, resulting in the formation of artemisinin–heme adducts and artemisinin–protein adducts that are toxic to plasmodia.

### Primaquine

**Primaquine** was approved in 1952 for the treatment of individuals with malaria. Because primaquine attacks the hepatic forms of malaria caused by *P. vivax* and *P. ovale*, it is used to prevent recrudescence of these infections. Primaquine severely disrupts the metabolic processes of plasmodial mitochondria. The antimalarial activity is probably attributable to **quinone,** a primaquine metabolite that interferes with the function of **ubiquinone** as an electron carrier in the respiratory chain. Other potential mechanisms of action include the generation of metabolites of primaquine that cause nonspecific oxidative damage to plasmodial mitochondria.

Individuals with **G6PD (glucose-6-phosphate dehydrogenase)** deficiency have a limited ability to protect their erythrocytes against oxidative damage. G6PD is needed to reduce $NADP^+$ to NADPH, which converts oxidized **glutathione** to reduced glutathione. Reduced glutathione protects erythrocytes by catalyzing the breakdown of toxic oxidant compounds. The administration of primaquine causes significant oxidative stress because of the formation of numerous oxidized compounds. As a result, primaquine can induce massive and potentially fatal **hemolysis** in individuals with G6PD deficiency. Primaquine should, therefore, never be administered to a person without first confirming adequate G6PD activity in that individual's erythrocytes. Primaquine should never be administered to pregnant women, because primaquine crosses the placenta and can induce fatal hemolysis in fetal erythrocytes independent of the maternal G6PD status. Primaquine can also cause gastrointestinal disturbances, methemoglobinemia and, very rarely, neutropenia, hypertension, arrhythmias, and CNS symptoms. Primaquine is predominantly used to clear hepatic hypnozoites from individuals with malaria caused by *P. vivax* or *P. ovale;* however, it is increasingly being used as a prophylactic agent as well.

### Atovaquone

**Atovaquone** is a structural analogue of ubiquinone, the shuttling protein in the electron transport chain. Under physiologic conditions, the transfer of two electrons to the cytochrome $bc_1$ complex in the electron transport chain (Fig. 37-3) oxidizes reduced ubiquinone. Atovaquone inhibits the interaction between reduced ubiquinone and the cytochrome $bc_1$ complex, thereby disrupting electron transport. Because plasmodia depend on the electron transport chain to regenerate oxidized dihydroorotate reductase, treatment with atovaquone disrupts pyrimidine synthesis and, thereby, prevents plasmodia from replicating their DNA. It is likely that inhibition of the electron transport chain also disrupts other steps in intermediary metabolism that rely on oxidation/reduction cycling of proteins.

The cytochrome $bc_1$ complex is a ubiquitous feature of eukaryotic organisms. The selectivity of atovaquone for plasmodia likely relies on differences in the sequences of amino acids in human and plasmodial ubiquinone–cytochrome $bc_1$ binding regions. Atovaquone inhibits the activity of the plasmodial isoform of cytochrome $bc_1$ with approximately 100-fold selectivity as compared to the human isoform. However, this selectivity is easily disrupted; plasmodia can develop resistance to atovaquone with a single point mutation in the cytochrome $bc_1$ complex. For this reason, atovaquone is not used as a single agent. Atovaquone can be coadministered with doxycycline or as a fixed combination with **proguanil,** an inhibitor of dihydrofolate reductase (see Chapter 31). Proguanil and atovaquone are synergistic in their antimalarial activity. Interestingly, this synergy does not seem to be related to proguanil's action as a folate synthesis inhibitor, because other inhibitors of dihydrofolate reductase do not have synergistic effects with atovaquone. When administered with atovaquone, proguanil may act as an uncoupling agent across mitochondrial membranes, thereby enhancing atovaquone-mediated mitochrondrial depolarization. Atovaquone is generally well tolerated, with a low incidence of gastrointestinal side effects and occasional rash. It can be used (in combination with another drug) both therapeutically and prophylactically.

## OTHER PROTOZOA

In addition to plasmodium, other medically important protozoa include *Entamoeba histolytica,* the organism that causes amebiasis; *Trypanosoma brucei rhodesiense* and *T. b. gambiense,* the causative agents of African sleeping sickness; *Trypanosoma cruzi,* the causative agent of Chagas' disease; and *Leishmania* spp., the causative agents of leishmaniasis. Because more is known about *Entamoeba histolytica,* the following physiology section focuses on this parasite; however, the pharmacology section includes not only discussions of agents effective against amebiasis, but also discussions of agents effective against African sleeping sickness, Chagas' disease, and leishmaniasis.

### Case 3

Mr. Smith, a 29-year-old American journalist, returns from a trip to Southeast Asia. He feels fine for 5 weeks, but then begins to experience mild diarrhea, abdominal pain, and malaise. He does not attribute his symptoms to the trip, because they developed well after he returned home. Furthermore, Mr. Smith's wife shared the same food and water during the trip, and she remains completely fine. As a result, Mr. Smith ignores the symptoms for a week, but he eventually goes to his physician when the symptoms do not abate spontaneously. Physical examination reveals tenderness in the right upper quadrant of the abdomen. Blood tests are notable for elevated liver enzymes, and a computed tomography scan reveals a liver abscess. Stool examination is positive for heme and for *E. histolytica* cysts.

### QUESTIONS

1. Why is Mr. Smith's wife asymptomatic?
2. What are the potential complications if Mr. Smith's condition is left untreated?

## PHYSIOLOGY OF LUMINAL PROTOZOA

The enteric protozoa *Entamoeba dispar* and *Entamoeba histolytica* are morphologically indistinguishable, although these two species can be differentiated using specific monoclonal antibodies. *E. dispar* does not cause invasive disease (i.e., it does not compromise the gut epithelium), but *E. histolytica* can cause an asymptomatic carrier state, invasive colitis, and/or so-called metastatic infections (usually hepatic abscesses). Because these two species have only recently been distinguished, conclusions drawn from the older literature must be interpreted with caution.

Five to ten percent of individuals who live in poverty in the developing world have serological evidence of previous *E. histolytica* infection. It is estimated that 50 million cases of dysentery are caused by *E. histolytica* occur each year, resulting in 40,000–100,000 deaths. Because Mr. Smith's wife shared the same food and water with her husband, she was also likely to be infected with *E. histolytica.* For unclear reasons, she excreted *E. histolytica* asymptomatically, while her husband developed invasive disease.

## Life Cycle of *Entamoeba histolytica*

Colonic infection with *Entamoeba histolytica* occurs as a result of ingestion of cysts through the fecal–oral route; for example, drinking contaminated water. Whether intestinal invasion occurs may be a function of the number of cysts ingested, the strain of the parasite, the motility of the host gastrointestinal tract, and the presence of appropriate enteric bacteria to serve as nourishment for the ameba. Disease results when active trophozoites invade the intestinal epithelium, and secondary spread to the liver can occur via the portal circulation (Fig. 37-6). As its name implies, *E. histolytica* lyses and destroys human tissue. Trophozoites typically multiply above the muscularis mucosae of the intestines and spread laterally. They may also penetrate more deeply, occasionally perforating the intestinal wall and spreading contiguously. Seeding of the liver is also common. In Mr. Smith's case, a CT scan revealed involvement of the liver with formation of an abscess.

*E. histolytica* exists in two forms: the inactive but infective **cyst** and the active **trophozoite.** Cysts are ingested in contaminated food or water. Excystation occurs in the small intestine, where the trophozoites mature. The trophozoite form is capable of invading host tissue. Inside the human body, the trophozoites move using pseudopods and ingest bacteria, other protozoa, and host intestinal and red blood cells. A trophozoite can convert to a binucleated cyst form, which matures into a tetranucleated cyst that travels through the colon but is not capable of mucosal invasion (Fig. 37-6).

Symptoms from amebiasis vary from diarrhea and abdominal cramps to fulminant dysentery and hepatic abscess formation. Fewer than 40% of individuals with amebic dysentery develop fever, and microscopic evaluation of the stool usually discloses few neutrophils. The onset of symptoms can range from a few days to a year after exposure, or symptoms may never occur. Mr. Smith's symptoms did not develop until at least a month after exposure, giving him reason not to attribute his symptoms to his travels.

## Fermentation Pathways

*E. histolytica* and other luminal parasites are diverse eukaryotes with novel adaptations to their anaerobic niche. For example, *E. histolytica* lack **fermentation enzymes** (lactate dehydrogenase and pyruvate decarboxylase) that are present in yeast and other eukaryotes. They also lack enzymes of oxidative phosphorylation, the Krebs cycle, and pyruvate dehydrogenase. Instead, ameba (and many anaerobic organisms) utilize novel enzymes that provide a source for the electron transfers that drive metabolism.

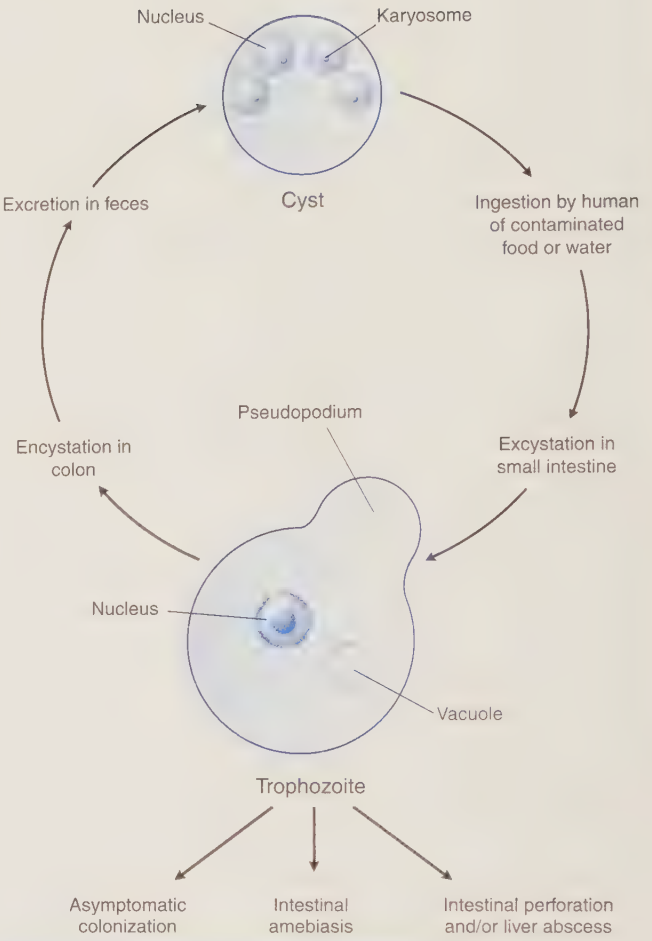

Nucleus
Karyosome
Cyst
Excretion in feces
Ingestion by human of contaminated food or water
Excystation in small intestine
Encystation in colon
Pseudopodium
Nucleus
Vacuole
Trophozoite
Asymptomatic colonization
Intestinal amebiasis
Intestinal perforation and/or liver abscess

**Figure 37-6. Manifestations of Amebiasis.** Ingestion of *Entamoeba histolytica* cysts can result in several different clinical outcomes, ranging from asymptomatic excretion of the cysts to invasive disease. Asymptomatic infection occurs when the ingested cysts excyst (mature) in the small intestine but do not invade the intestinal mucosa. These trophozoites then encyst in the colon, and excretion occurs in the feces. Invasive disease results when active trophozoites invade the intestinal epithelium. This invasion can result in asymptomatic colonization, intestinal amebiasis (amebic dysentery)—which is characterized by diarrhea and abdominal cramps—or intestinal perforation. Spread of infection via the portal vein can cause liver abscesses.

*Ameba are obligate fermenters of glucose to ethanol* (Fig. 37-7). Many of these fermentation enzymes, which are absent from humans, yeast, and most eubacteria, contain a set of iron-sulfur centers called **ferredoxins** that transfer electrons under strongly reducing (anaerobic) conditions. This is in contrast to heme and cytochromes, which use iron centers to transfer electrons under oxidizing (aerobic) conditions. Pyruvate:ferredoxin oxidoreductase (**PFOR**), which contains a single ferredoxin domain, catalyzes the decarboxylation of pyruvate to acetyl CoA, with the production of $CO_2$. PFOR activity also produces reduced ferredoxin, which can, in turn, reduce protons to form hydrogen gas or reduce $NADP^+$ to

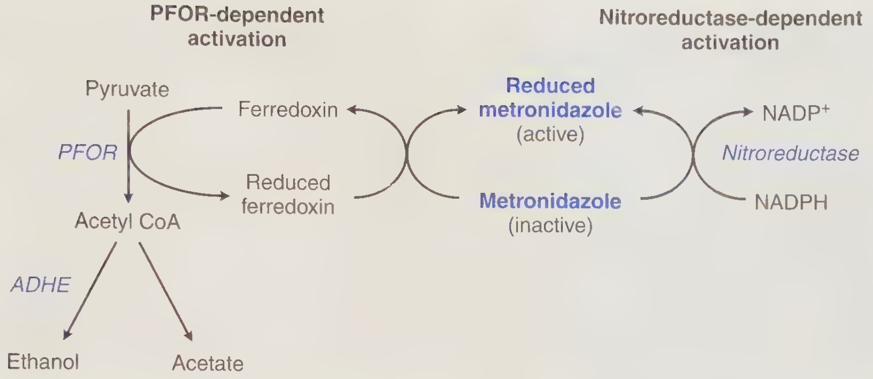


**Figure 37-7. Fermentation Enzymes of Anaerobic Organisms and Mechanisms of Metronidazole Activation.** Anaerobic organisms metabolize pyruvate to acetyl CoA; this conversion is catalyzed by the enzyme pyruvate:ferredoxin oxidoreductase (PFOR). Acetyl CoA is then either hydrolyzed to acetate or oxidized to ethanol by alcohol dehydrogenase E (ADHE). Metronidazole is a prodrug; it contains a nitro group that must be reduced for the drug to become active. Reduced metronidazole is highly effective against anaerobic organisms, probably because of the formation of cytotoxic intermediates that cause DNA, protein, and membrane damage. Two aspects of anaerobic metabolism provide opportunities for selective reduction of the nitro group. First, the reaction catalyzed by PFOR results in the reduction of ferredoxin; reduced ferredoxin can then transfer its electrons to metronidazole, resulting in reduced (active) metronidazole and reoxidized ferredoxin. Second, many anaerobic organisms express nitroreductase enzymes that selectively reduce metronidazole and, in the process, oxidize NADPH to $NADP^+$.

NADPH. Acetyl CoA is reduced to ethanol via alcohol dehydrogenase E (ADHE), with the recovery of two $NAD^+$ cofactors. Anaerobic bacteria (e.g., *Helicobacter* spp. and *Clostridia* spp.) express PFORs, ferredoxins, and ADHEs similar to those of luminal protozoa. Indeed, phylogenetic analyses suggest that most of the genes encoding parasite fermentation enzymes, and many of the genes encoding parasite enzymes involved in core energy metabolism, have been laterally transferred from anaerobic bacteria. Although lateral gene transfer is extraordinarily frequent between bacteria, it is extremely rare between bacteria and higher eukaryotes, which maintain their gametes in a sterile environment.

# PHARMACOLOGY OF ANTI-PROTOZOAL AGENTS

## Metronidazole

Metronidazole is inactive until it is reduced within host or microbial cells possessing a large negative redox potential, such as in anaerobic or microaerophilic luminal parasites. Activation can occur by interaction with reduced ferredoxin or with specific nitroreductases (Fig. 37-7). Activated metronidazole forms reduced cytotoxic compounds that bind to proteins, membranes, and DNA in target cells, causing severe damage.

*Metronidazole sensitivity is directly related to the presence of PFOR activity.* Most eukaryotes and eubacteria lack PFOR and, therefore, fail to activate metronidazole. However, in poorly oxygenated tissues such as abscesses, metronidazole can be activated. Because PFOR is expressed in protozoa but has no counterpart in mammalian systems, the drug is selectively toxic for ameba and anaerobic organisms.

The widespread use of metronidazole has led to drug resistance in *Helicobacter pylori,* a common bacterial cause of gastritis, which, if left untreated, can lead to peptic ulcers and gastric malignancy. This resistance is due to a null mutation in the *rdxA* gene, which encodes an oxygen-insensitive NADPH nitroreductase. Low-level resistance to metronidazole has also been observed in a number of anaerobic protozoa, including trichomonads (caused by decreased expression of ferredoxin), giardia (caused by decreased PFOR activity and decreased drug permeability), and ameba (caused by increased expression of **superoxide dismutase**). Metronidazole resistance among luminal parasites has, however, not yet become clinically important.

There are three explanations for the slow development of resistance to metronidazole among luminal parasites. First, luminal parasites are generally diploid, so a single mutation will not usually confer resistance. This contrasts with the case of haploid bacteria and certain haploid stages of *P. falciparum,* in which resistance develops more quickly. Second, luminal parasites have few metabolic alternatives to PFOR activity. Third, metronidazole is hydrophilic, so overexpression or modification of P-glycoprotein, which confers resistance to hydrophobic drugs, does not increase steady-state metronidazole efflux.

Side effects of metronidazole include gastrointestinal discomfort, headaches, occasional neuropathy, a metallic taste, and nausea. Metronidazole also causes nausea and flushing when taken concomitantly with alcohol (a so-called disulfiram-like effect, caused by inhibition of ethanol metabolism). Metronidazole is active against *E. histolytica* trophozoites in tissues, but has much less activity against intraluminal ameba (probably, in large part, because of metronidazole's excellent absorption in the upper gastrointestinal tract and, consequently, its low drug levels in the lumen of the colon, where the ameba live). Individuals with invasive amebiasis are, therefore, typically first treated with metronidazole (to eradicate trophozoites that are actively invading human tissue), and then treated with a second agent that has more intraluminal activity, such as **iodoquinol** or **paromomycin.** The latter two agents kill ameba by unknown mechanisms, but are poorly absorbed from the intestinal tract and, therefore, reach high concentrations in the lumen of the colon.

## Other Anti-Protozoal Agents

**Pentamidine** can be used to treat individuals with early-stage African trypanosomiasis (African sleeping sickness), which is caused by ***Trypanosoma brucei gambiense*** and certain strains of ***T. b. rhodesiense.*** Early-stage disease is defined as disease that does not yet involve the central nervous system. Pentamidine inhibits DNA, RNA, protein, and phospholipid synthesis. The drug has a high affinity for DNA in kinetoplasts (a DNA-containing organelle in certain protozoa), and it suppresses kinetoplast replication and function. Kinetoplastidan protozoa include *Trypanosoma* and *Leishmania* spp. Pentamidine may also inhibit **dihydrofolate reductase** activity. Some strains of *Trypanosoma* have a high-affinity uptake system for the drug, contributing to its selectivity. Pentamidine can cause fatigue, dizziness, hypotension, pancreatitis, and kidney damage. Pentamidine is now most commonly used as a second-line treatment for individuals with ***Pneumocystis carinii* pneumonia** (PCP), a common infection in patients with AIDS.

**Suramin** is another drug used to treat individuals with early-stage African trypanosomiasis. Suramin combines readily with many macromolecules and inhibits numerous enzymes, including those involved in energy metabolism (e.g., glycerol phosphate dehydrogenase). It also inhibits RNA polymerase and, thus, interferes with parasite replication. Suramin can cause pruritus, paresthesias, vomiting, and nausea. The biochemical basis for suramin's relative selectivity for African trypanosomiasis is not well understood.

**Melarsoprol** is a front-line drug used to treat many individuals with late-stage African trypanosomiasis (i.e., disease that involves the central nervous system). Melarsoprol was developed by adding the heavy-metal chelator dimercaptopropanol to the trivalent arsenic of melarsen oxide. The drug is insoluble in water, and is instead dissolved in propylene glycol. Blood trypanosomes lack a functional tricarboxylic acid cycle and are entirely dependent on glycolysis for ATP production. Melarsoprol inhibits trypanosomal pyruvate kinase, thereby inhibiting glycolysis and decreasing production of ATP. Affected trypanosomes quickly lose motility and lyse. Melarsoprol also inhibits the uptake of adenine and adenosine by trypanosomal transporters. Mammalian cells are less permeable to the drug than are trypanosomes, so the drug has some selectivity. Unfortunately, melarsoprol is still quite toxic to humans (4–6% death rate). Melarsoprol is administered intravenously and can cause severe phlebitis. It is also corrosive to plastics, limiting storage and administration options. In addition, 5–10% of individuals with late-stage African trypanosomiasis develop intense inflammation of the brain after administration of melarsoprol ("reactive encephalopathy"); this complication is associated with a mortality of greater than 50%. Concomitant administration of steroids lessens the likelihood of reactive encephalopathy. Polyneuropathy after melarsoprol administration is also common (10%), and can be lessened by concomitant administration of thiamine.

A much less toxic alternative to melarsoprol in the treatment of individuals with African trypanosomiasis caused by *T. b. gambiense* (West Africa sleeping sickness) is **eflornithine** (α-difluoromethylornithine). Eflornithine is highly effective against both early- and late-stage West African sleeping sickness, but not against East African trypanosomiasis (caused by *T. b. rhodesiense*). Eflornithine is a selective and irreversible inhibitor of ornithine decarboxylase and, thus, of polyamine synthesis. Ornithine decarboxylase converts ornithine to putrescine; this is a rate-limiting step in the synthesis of putrescine and the polyamines spermine and spermidine. Polyamines are involved in nucleic acid synthesis and the regulation of protein synthesis. *T. b. gambiense* organisms are susceptible to eflornithine, possibly because of the slow turnover of ornithine decarboxylase in these parasites, but *T. b. rhodesiense* have a higher rate of turnover (as do human cells) and are intrinsically less sensitive.

**Nifurtimox** is used in the treatment of individuals with New World trypanosomiasis (Chagas's disease), which is caused by ***Trypanosoma cruzi.*** The drug undergoes reduction and generates toxic intracellular oxygen radicals in the parasite. It first forms reduced intermediates, such as nitroaryl radicals. These radicals can then be oxidized to generate **superoxide anions,** which react with water to produce cytotoxic hydrogen peroxide. Some parasites, such as trypanosomes, lack **catalase** and other enzymes capable of degrading hydrogen peroxide. Such parasites are, thus, sensitive to the toxicity of nitroaromatic drugs. Mammalian cells are protected because of the presence of such antioxidant enzymes as catalase, glutathione peroxidase, and superoxide dismutase. Nifurtimox can cause anorexia, vomiting, memory loss, sleep disorders, and convulsions.

**Sodium stibogluconate** and **meglumine antimonate** are used to treat individuals with leishmaniasis, which is caused by parasites of the genus *Leishmania.* These agents contain pentavalent antimony and work by an unknown mechanism. It is postulated that these drugs inhibit the glycolytic pathway and fatty acid oxidation, processes that are crucial for intermediary metabolism. The pentavalent antimony can also have many nonspecific effects, such as modification of sulfhydryl groups. These drugs can cause bone marrow suppression, a prolonged QT interval, pancreatitis, and rash.

Resistance of leishmania to antimonial agents is being recognized with increasing frequency, especially in South Asia. Alternative agents include **amphotericin** and **miltefosine.** The mechanism of action of miltefosine is unknown. It is a synthetic ether phospholipid analogue that is chemically similar to natural phospholipids present in cell membranes. Miltefosine has been shown to have antineoplastic, immunomodulatory, and antiprotozoal activity. It is presumed that the cytostatic and cytotoxic effects of miltefosine are caused by inhibition of enzyme systems in cell membranes (such as protein kinase C) and inhibition of phosphatidylcholine biosynthesis. Miltefosine may also inhibit platelet activating factor-induced responses and inositol phosphate formation. The immunomodulatory effects of miltefosine include increased T-cell activation, interferon-gamma production in peripheral mononuclear cells, and increased interleukin-2 receptor and HLA-DR antigen expression. The drug can be administered orally, and can be used in the treatment of individuals with visceral leishmaniasis.

## HELMINTHS

Helminths are multi-cellular worms with digestive, excretory, nervous, and reproductive systems. Parasitic helminths can infect the liver, blood, intestines, and other tissues in human hosts. Clinically significant worms can be divided phylogenetically into three classes: **nematodes** (roundworms), **trematodes** (flukes), and **cestodes** (tapeworms). The presence of a rudimentary nervous system provides a number of possible targets for antihelminthic agents. The physiology of ***Onchocerca volvulus,*** which causes onchocerciasis ("river blindness"), provides an example of potential targets for antihelminthic drugs. Although the majority of the following discussion focuses on the physiology and pharmacology of onchocerciasis, a number of other antihelminthic agents are also presented.

### Case 4

Thumbi is a boy who enjoys fishing in a river near his village in the Democratic Republic of Congo. At the age of 13, he emigrates with his family to the United States. Shortly thereafter, he begins to scratch his arms and legs vigorously. Six months later, his mother brings him to a dermatologist. Physical examination reveals a macular and papular rash with excoriations on the arms and legs, as well as a few subcutaneous nodules. Examination of peripheral blood discloses high-level eosinophilia. A nodule is excised and examined by a pathologist, leading to a diagnosis. Thumbi begins treatment with ivermectin, but returns the next day feverish and feeling more itchy than before.

## QUESTIONS

1. What did the pathologist see in the nodule?
2. Why did Thumbi feel worse immediately after treatment with ivermectin?

## PHYSIOLOGY OF HELMINTHS

Humans can become infected with helminths when they ingest food or water contaminated with eggs or **larvae.** In addition, larvae in soil can directly penetrate human skin, and insects can transmit still other larvae through bites. If humans are the definitive host, the eggs or larvae develop in humans into adult worms that can migrate through tissues and enter the sexual stage. During the sexual stage, adult worms release additional eggs or larvae, which can then pass out of the host through the gastrointestinal or urinary tracts. Larvae in humans can also be ingested by insects during a blood meal. In the environment or within vector hosts, eggs or larvae then become infective for humans, and the cycles start over.

### Life Cycle of *Onchocerca volvulus*

Onchocerciasis is one of eight human filarial infections (a specific type of nematodal worm infection). In Thumbi's case, an infected *Simulium* spp. blackfly bit and inoculated *O. volvulus* larvae into his skin in Africa. Adult worms then developed in Thumbi's subcutaneous tissues. These adult male and female filarial worms came to rest in subcutaneous nodules, in which they mated (Fig. 37-8). Adult worms are large (3–80 cm in length), look like spaghetti or angel hair pasta, and can live for

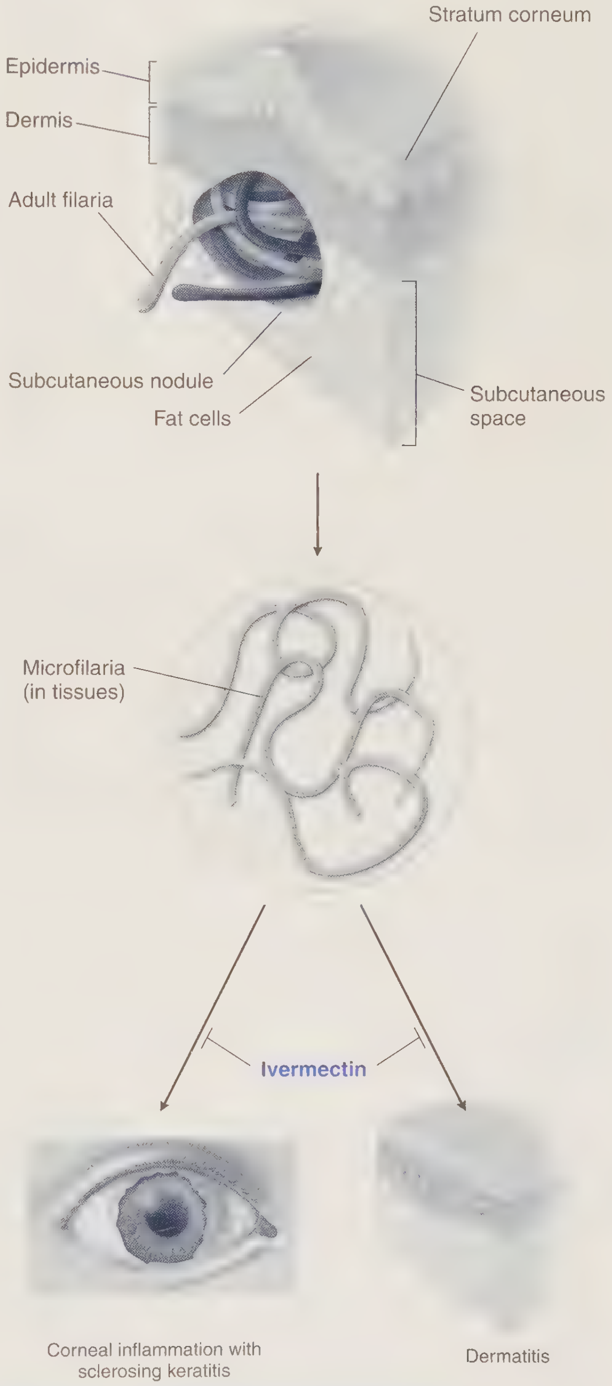


**Figure 37-8. Life Cycle of *Onchocerca volvulus*.** Adult filarial worms mate in subcutaneous nodules in humans, releasing microfilariae that migrate through the skin and subcutaneous tissues, causing dermatitis and pruritus. Microfilariae migrating through the eye induce ocular inflammation, which can lead to corneal scarring and blindness ("river blindness"). Ivermectin, the agent of choice for treating individuals with onchocerciasis, is effective only against microfilariae; the drug does not kill adult filarial worms.

10–15 years. The nodules have a characteristic appearance that was recognized by the pathologist. From these nodules ("onchocercomata"), gravid females release millions of microfilariae, which migrate freely through the skin and cornea. If ingested by a *Simulium* fly, additional maturation can occur and the cycle can continue. The diagnosis of onchocerciasis is usually based on microscopic detection of microfilariae in skin snips, not on pathological examination of excised onchocercomata. Microfilariae are small (200–400 μm); as they degenerate and die, they cause local inflammatory reactions, provoking itching, dermatitis, and eventually scarring. When microfilariae die in the cornea, they induce a punctate keratitis that, over years, leads to scarring and blindness. Such ocular involvement has made onchocerciasis the second leading cause of infectious blindness in the world (after trachoma), and is the reason onchocerciasis is also referred to as "river blindness" (also reflecting the fact that the blackflies carrying the larvae inhabit flowing streams, such as the one in which Thumbi enjoyed fishing). Without treatment, Thumbi could well become one of the 770,000 people in the world who are currently blind or visually impaired from onchocerciasis.

## Neuromuscular Activity

The subcuticular layer of longitudinal muscle in nematodes is inhibited by **GABAergic** and excited by **cholinergic** transmitters. The motor neurons of invertebrates are unmyelinated, making them more vulnerable to neurotoxins than the myelinated somatic motor neurons of humans. (See Chapter 6, Principles of Nervous System Physiology and Pharmacology, for more information on the human nervous system.) Many anti-helminthic agents modulate parasite neuromuscular activity by enhancing inhibitory signaling, antagonizing excitatory signaling (nondepolarizing block), or tonically stimulating excitatory signaling (depolarizing block).

# PHARMACOLOGY OF ANTI-HELMINTHIC AGENTS

## Agents That Interrupt Neuromuscular Activity

### Ivermectin

**Ivermectin** is a semisynthetic macrocyclic lactone that acts against a broad range of helminths and arthropods and that has been used most extensively to treat and control onchocerciasis. Ivermectin's exact mechanism of action is unclear, but studies in *Caenorhabditis elegans* (a soil helminth that is studied extensively in eukaryotic biology as a simple model organism) suggest that the mechanism of action involves potentiation and/or direct activation of **glutamate-gated chloride channels** in nematode cell membranes. This results in hyperpolarization of neuromuscular cells and pharyngeal paralysis. Ivermectin is also thought to affect **gamma-aminobutyric acid (GABA)** inhibitory transmission by potentiating the release of GABA from presynaptic terminals, directly activating GABA receptors, and potentiating the binding of GABA to its receptor. All of these effects increase GABA-mediated transmission of signals in peripheral nerves, resulting in hyperpolarization. *The ultimate result is blockade of neuromuscular transmission and tonic paralysis of the worm.*

Pharyngeal paralysis of *O. volvulus* inhibits nutrient uptake and kills developing larvae (microfilariae). Unfortunately, ivermectin does not kill adult filarial worms. It does, however, destroy microfilariae in utero, thereby preventing production and release of new microfilariae from adult female worms for at least 6 months. Thus, ivermectin is used to prevent microfilaria-mediated ocular damage, and to decrease human-to-vector transmission (because microfilariae are infectious to simulial flies), but it cannot cure human hosts of *O. volvulus* infection. Because the drug is noncurative, it is typically administered to infected humans every 6 months for the life expectancy of the adult worms (10–15 years).

Ivermectin does interact with GABA receptors in vertebrates, but its affinity for invertebrate GABA receptors is about 100 times greater. Cestodes and trematodes lack high-affinity ivermectin receptors, which may explain why these organisms are not sensitive to the drug. GABA receptors in humans are also generally present in the central nervous system, but because ivermectin does not cross the blood–brain barrier, the drug is generally well tolerated in humans. When the blood–brain barrier is hyperpermeable, as in patients with meningitis, ivermectin can be more toxic, and can result in headaches, ataxia, and coma. Side effects of ivermectin are usually attributable to inflammatory or allergic responses to dying microfilariae ("Mazzotti-type reaction"), and include headaches, dizziness, weakness, rash, pruritus, edema, abdominal pain, hypotension, and fever. This is why Thumbi felt worse the day after initiation of his treatment.

Ivermectin is widely used to treat animals with nematode infections, and resistance to ivermectin in livestock parasites is already recognized. Fortunately, clinically important resistance in humans has not yet been documented. The P-glycoprotein may be involved in ivermectin resistance, although the exact mechanism of resistance has not yet been elucidated. In studies of mice, disruption of the *mdr1a* gene, which encodes a P-glycoprotein membrane transporter, results in hypersensitivity to ivermectin. Furthermore, analysis of P-glycoprotein cDNA from *Haemonchus contortus* (a nematode of veterinary importance) shows 65% homology to P-glycoprotein/multidrug resistant protein sequences in mice and humans. P-glycoprotein mRNA expression is higher in ivermectin-selected strains of *H. contortus* than in unselected strains, and verapamil, which reverses multidrug resistance by blocking P-glycoprotein channels, increases the efficacy of ivermectin.

In addition to its use in the treatment of individuals with onchocerciasis, ivermectin is also used to treat individuals with strongyloidiasis and cutaneous larva migrans (both are nematodal infections) and individuals with scabies (an ectoparasitic infestation).

### Piperazine

**Piperazine** is an anti-helminthic agent of historical interest that was previously used to treat individuals infected with the nematodes *Ascaris lumbricoides* ("roundworm") and *Enterobius vermicularis* ("pinworm"). More effective agents have now replaced piperazine. Piperazine acts as a GABA receptor agonist; it increases the flux of hyperpolarizing chloride ions across nematode membranes, resulting in flaccid paralysis. The mechanism of piperazine selectivity for nematodes is unknown, but it is thought that the drug could selectively disrupt nematodal pacemaker cells that drive rhythmic muscle contraction. Piperazine is generally well tolerated, but can occasionally cause gastrointestinal distress, pruritus, and seizures.

### *Pyrantel Pamoate*

**Pyrantel pamoate** is another agent of predominantly historical interest. It is still used sometimes as a third-line agent to treat individuals with hookworm, pinworm, or roundworm infection. Pyrantel pamoate acts as an agonist at cation channels, resulting in sustained membrane depolarization and tonic paralysis. Evidence suggests that this drug may also act as an acetylcholinesterase inhibitor, an effect that would amplify membrane depolarization. Side effects include gastrointestinal distress and dizziness. At supratherapeutic doses, pyrantel pamoate can cause systemic neuromuscular blockade.

## Other Anti-Helminthic Agents

**Albendazole, mebendazole, and thiabendazole** inhibit tubulin polymerization by binding to β-tubulin. Evidence suggests that these agents are selective for the nematodal isoform of β-tubulin, thus decreasing host toxicity. Inhibition of tubulin polymerization disrupts nematodal motility and DNA replication (see Chapter 32, Pharmacology of DNA Replication and Mitosis), leading to degenerative changes in integumental and intestinal cells of helminths, and eventually causing immobilization and death of the worms. The effects of the drugs against immobile tissue forms of cestodal larval parasites (e.g., cysticercosis and echinococcosis) are less well understood, but may also involve β-tubulin binding and disruption of the integumental integrity of the protoscolex, a larval structure that eventually becomes the "head" of the adult cestode. Thiabendazole causes significant nausea, vomiting, and anorexia at therapeutic doses, and is rarely used. Mebendazole and albendazole are better tolerated; albendazole has the highest bioavailability after oral administration.

**Praziquantel** is the drug of choice for treating individuals infected with adult cestode (tapeworm) and trematode (fluke) infections. Specifically, praziquantel is the drug of choice for treating individuals with schistosomiasis, a trematodal infection that causes considerable morbidity and mortality worldwide. Although the exact mechanism of praziquantel's action is unknown, it seems to increase parasite membrane permeability to calcium, resulting in contraction and paralysis of the worms. The main side effects of praziquantel include nausea, headache, and abdominal discomfort.

**Diethylcarbamazine** (DEC), a piperazine derivative, is the drug of choice for treating individuals with certain filarial infections, including lymphatic filariasis. Its use in the treatment of individuals with filarial onchocerciasis has been largely supplanted by the use of ivermectin (predominantly because of ivermectin's improved tolerability and ease of administration). Unlike ivermectin, however, DEC kills adult filarial worms and is, thus, a curative agent. DEC's mechanism of action is unknown; current hypotheses include stimulation of innate immune mechanisms, inhibition of microtubule polymerization, and inhibition of arachidonic acid metabolism. DEC is reasonably well tolerated at low doses; its major side effects include anorexia, headache, and nausea. Administration of DEC can, however, precipitate Mazzotti reactions in individuals with heavy microfilarial burdens, and such reactions can be fatal. Administration of gradually increasing doses of DEC minimizes this possibility. DEC is excreted by the kidneys, and dosing may need to be adjusted in individuals with decreased renal function.

Antibacterial agents may also have a role in treating individuals with certain helminthic infections. For example, *O. volvulus* has been found to contain an obligate symbiont (*Wolbachia* endobacteria) important in helminth fertility. Use of doxycycline to treat individuals with onchocerciasis leads to decreased fertility, embryogenesis and viability of *O. volvulus*.

## *Conclusion and Future Directions*

The development of new antiparasitic agents will rely on continued exploitation of molecular and metabolic differences between parasites and hosts. Recent advances in the application of molecular biological and genetic techniques to study parasitic eukaryotes, and detailed knowledge of parasite, vector, and host genomes, transcriptomes, and proteomes, should facilitate the development of new agents effective against many parasitic infections.

## *Suggested Readings*

Francis SE, Sullivan DJ Jr, Goldberg DE. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum.* Ann Rev Microbiol 1997;51:97–123. (*Current understanding of hemoglobin metabolism in the food vacuole.*)

Gardner MJ, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum.* Nature 2002;419:498–511. (*Discusses the various molecular targets that can be pursued now that the* P. falciparum *genome has been completed.*)

Hoerauf, A, Mand S, Volkmann L, et al. Doxycycline in the treatment of human onchocerciasis: Kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female *Onchocerca* worms. Microbes Infect 2003;5:261–273. (*Reports antibacterial treatment for onchocerciasis.*)

Jeong JY, Mukhopadhyay AK, Akada JK, et al. Roles of FrxA and RdxA nitroreductases of *Helicobacter pylori* in susceptibility and resistance to metronidazole. J Bacteriol 2001;183:5155–5162. (*Discusses the growing epidemic of* H. pylori *resistance to metronidazole.*)

Sidhu ABS, Verdier-Pinard D, Fidock DA. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcrt* mutations. Science 2002;298:210–213. (*A possible molecular explanation for malarial drug resistance.*)

Upcroft P, Upcroft J. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev 2001;14:150–164. (*Discusses mechanisms of resistance to metronidazole; predicts new targets for anti-protozoal agents.*)

## Drug Summary Table Pharmacology of Parasitic Infections

| Drug | Clinical Use | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ANTIMALARIAL AGENTS: INHIBITORS OF HEME METABOLISM**<br>*Mechanism—↓ metabolism/removal of toxic heme → ↑ toxicity in plasmodia* | | | | |
| **Chloroquine** | Malaria, all species | Pruritus, retinopathy | Psoriasis, porphyria | Kills only erythrocytic stage of plasmodial infections<br>Used prophylactically and therapeutically<br>Commonly used in suicides |
| **Quinine** | Malaria, especially *P. falciparum* | Tinnitus, headache, nausea, deafness | Tinnitus | Used for treatment; not used prophylactically |
| **Mefloquine** | Chloroquine-resistant malaria | Nausea, vomiting, diarrhea, neuropsychiatric changes, cardiac conduction changes | Seizures, arrhythmias, neuropsychiatric disorders | Used predominantly for prophylaxis |
| **Artemisinin** | Malaria, all species | Possible neurotoxicity<br>Prolonged QT interval | | Forms toxic adducts with heme, leading to cell damage<br>Frequently used in Africa and Asia, often in combination with a second agent<br>Not used prophylactically<br>Not currently available in the United States |
| **ANTIMALARIAL AGENTS: INHIBITORS OF THE ELECTRON TRANSFER CHAIN**<br>*Mechanism—See text* | | | | |
| **Primaquine** | *P. vivax, P. ovale* | Hemolytic anemia, intestinal distress, methemoglobinemia | Glucose-6-phosphate dehydrogenase deficiency, pregnancy | Kills both liver and erythrocyte stage malarial parasites<br>Damages plasmodial mitochondria (mechanism unknown)<br>Used to eradicate hypnozoites (radical cure) of *P. vivax* and *P. ovale;* sometimes used as primary prophylaxis against all malarial plasmodia |
| **Atovaquone** | *P. falciparum*, toxoplasmosis, babesiosis | GI distress, headache, ↑ liver transaminases | | Used in combination with proguanil or doxycycline<br>Inhibits interaction of ubiquinone with cytochrome $bc_1$ complex |

**ANTI-PROTOZOAL AGENTS**

***Mechanism—Various, see Notes.***

| | | | | |
|---|---|---|---|---|
| **Metronidazole** | Amebiasis, giardiasis, trichomoniasis | Headache, nausea, metallic taste, neuropathy | Ethanol (disulfiram-like effect) | Activated by anaerobic parasite enzymes to form reduced cytotoxic compounds that bind to parasite proteins, membranes, DNA |
| **Pentamidine** | African trypanosomiasis, *Pneumocystis carinii* (*jiroveci*) pneumonia | Fatigue, dizziness, pancreatitis, hypotension, kidney damage | | Inhibits DNA, RNA, protein, and phospholipid synthesis and dihydrofolate reductase activity<br>Used in early-stage African trypanosomiasis |
| **Suramin** | African trypanosomiasis | Nausea, vomiting, shock | | Inhibits RNA polymerase and glycerol phosphate dehydrogenase<br>Used in early-stage African trypanosomiasis |
| **Melarsoprol** | African trypanosomiasis | Fever, phlebitis, neuropathy, reactive encephalopathy | | Inhibits pyruvate kinase → ↓ glycolysis → ↓ ATP<br>Inhibits uptake of adenine and adenosine<br>Mammalian cells are less permeable to drug<br>Used in late-stage African trypanosomiasis<br>Administration of drug associated with 4–6% death rate |
| **Eflornithine** | West African trypanosomiasis | | | Effective against early- and late-stage infection<br>Not effective against many strains of East African sleeping sickness |
| **Sodium stibogluconate**<br>**Meglumine antimonate** | Leishmaniasis | Bone marrow suppression, chemical pancreatitis, prolonged QT, rash | | Pentavalent antimonial compounds<br>May cause serum sickness-like reaction |
| **Miltefosine** | Visceral leishmaniasis | | | Immunomodulatory and anti-protozoal activity |
| **Nifurtimox** | Chagas' disease | Anorexia, vomiting, neuropathy, pancytopenia | | Becomes activated → generates toxic oxygen radicals in parasite<br>Mammalian cells are protected due to presence of catalase |

*(Continued)*

**Drug Summary Table**

## Pharmacology of Parasitic Infections *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **ANTI-HELMINTHIC AGENTS**<br>*Mechanism—Various, see Notes. All mechanisms lead to paralysis and death of worms.* | | | | |
| **Ivermectin** | Onchocerciasis, lymphatic filariasis, strongyloidiasis, scabies, cutaneous larva migrans | Itching, fever, headache, mostly due to dying parasites | Use with caution if blood–brain barrier is compromised | Hyperpolarization of parasite neuromuscular cells → tonic paralysis<br>Rare Mazzotti-type reactions |
| **Albendazole**<br>**Mebendazole**<br>**Thiabendazole** | Many nematode infections, cysticercosis, echinococcosis | Anorexia, nausea | | Inhibits tubulin polymerization → degenerative changes in integumental and intestinal cells of helminths → immobilization and death of parasite<br>Thiabendazole rarely used |
| **Diethylcarbamazine** | Filariasis | Anorexia, headache, nausea | Dosage reduction for patients with renal insufficiency | Mechanism of action unknown<br>May precipitate Mazzotti reaction |
| **Praziquantel** | Schistosomiasis, tapeworm infections, liver fluke infections | Nausea, headache, abdominal discomfort | | Increases parasitic cell membrane permeability to calcium → contraction and tonic paralysis of worms |
| **Pyrantel pamoate** | Pinworm, roundworm and hookworm infections | GI upset, dizziness | | Causes constant release of acetylcholine (ACh) → persistent activation of parasite nicotinic ACh receptors → tonic paralysis<br>Has been largely replaced by more effective and better tolerated agents |
| **Piperazine** | Roundworm infection | GI irritation, pruritus | Seizures | Rarely used; GABA agonist → flaccid paralysis |

# 38

# Principles of Combination Chemotherapy

Ryan L. Albritton, Donald M. Coen, and David E. Golan

**Introduction**
**Antimicrobial Combination Therapy**
  Minimum Inhibitory Concentration and Minimum Bactericidal Concentration
  Types of Drug Interactions—Synergy, Additivity, and Antagonism
  Examples of Antimicrobial Combination Therapy
    *Tuberculosis*
    *Synergistic Combinations*
    *Coadministration of Penicillins with ß-Lactamase Inhibitors*
    *Polymicrobial and Life-Threatening Infections*
  Unfavorable Drug Combinations
**Antiviral Combination Therapy: HIV**
**Antineoplastic Combination Chemotherapy**
  General Considerations
  Rationale for Combination Chemotherapy
  Examples of Antineoplastic Combination Chemotherapy
    *Hodgkin's Disease*
    *Testicular Cancer*
  Treatment of Refractory or Recurrent Disease
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Upon identifying the infectious agent or cell type responsible for an infectious or neoplastic illness, the physician may choose a single-drug therapy that specifically and potently targets that agent or cell type. Provided that the pathogen or tumor is susceptible, the emergence of resistance is rare, and the therapeutic index is high, such monotherapies, as compared to multidrug combinations, can minimize unwanted side effects. However, when pathogens or tumors are resistant to some chemotherapeutic agents and quickly develop resistance to others, when multiple pathogens with different drug susceptibilities are present simultaneously, or when the dose of the therapeutic agent is limited by toxicity, single-drug regimens often fail. Under these circumstances, combination chemotherapy may offer decisive advantages over monotherapy. The drugs in a multidrug regimen can interact synergistically to enhance the antimicrobial or antineoplastic potency of the combination, or can decrease the likelihood that resistance will emerge. Combinations are frequently used to reduce toxicity when individual drugs have low therapeutic indices, or when treatment must be initiated before the identification of the pathogen. Although combination chemotherapy opens new avenues for the expedient elimination of a pathogen or tumor from the body, it also introduces an extra level of complexity, with the potential for multiple side effects and drug interactions. The goal of any combination drug regimen should be to enable the efficient removal of the offending pathogen or tumor without incurring unacceptable toxicity to the host.

## ANTIMICROBIAL COMBINATION THERAPY

Microbial infections are commonly treated with drug combinations for a variety of reasons, including the threat of drug resistance, the need to treat immunocompromised patients, and the polymicrobial nature of many infections. Because microbes are genetically distant from humans, antimicrobial drug combinations can also offer the advantage of targeting several different molecular targets specific to the microbe(s), without a concomitant increase in side effects. This rationale is in stark comparison to the use of many antineoplastic drugs (see below), where side effects typically limit the dose of an agent. The following discussion provides conceptual background on the different types of antimicrobial drug interactions, then discusses specific examples of antimicrobial combination therapy.

## MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION

Antimicrobial agents with activity against a particular pathogenic microorganism (bacterial, protozoal, or fungal) can be characterized by the **minimum inhibitory concentration** (MIC) and the **minimum bactericidal concentration** (MBC) for that drug–pathogen pair. The MIC is defined as the lowest concentration of drug that inhibits growth of the microorganism after 18–24 hours of incubation in vitro. The MBC is defined as the lowest concentration of drug at which 99.9% of a culture of bacteria or some other microorganism is killed after 18–24 hours of incubation in vitro. It is important to note that the MIC and MBC refer to a specific drug–microbe pair and that, for a particular drug, these values may differ from one microbe to the next. Although the MBC is usually greater than the MIC, these quantities allow antimicrobial drugs to be grouped broadly into two categories: *cidal* and *static* (Table 38-1). An antimicrobial agent is *static* (e.g., bacterio*static,* fungi*static*) if its MBC is much greater than its MIC, and is *cidal* (e.g., bacteri*cidal,* fungi*cidal*) if its MBC is close to its MIC. In other words, *at therapeutic concentrations, cidal drugs kill the microorganism, while static drugs merely arrest microbial growth.* For example, most inhibitors of bacterial cell wall synthesis are cidal, whereas most inhibitors of bacterial protein synthesis are static (see Chapter 33, Pharmacology of Transcription and Translation, and Chapter 35, Pharmacology of the Bacterial Cell Wall). This distinction is admittedly somewhat artificial because many drugs with activity against an organism are bacteriostatic in one growth medium but bactericidal in another growth medium, or are bactericidal at sufficiently high concentrations. The principle is useful clinically, however, because such high concentrations are often not readily achievable due to dose-related toxicities and pharmacokinetic limitations (especially bioavailability and elimination) of the drug.

An important distinction between static and cidal drugs lies in their clinical applications. In general, the successful use of static drugs to treat infections requires an intact host immune system. This is because static drugs do not themselves kill existing microorganisms, but only prevent them from multiplying. Accordingly, such drugs rely on the host's immune and inflammatory mechanisms to effect clearance of existing organisms from the body. As one might expect, these drugs are more efficacious when initiated early in the course of an infection, when the infectious burden is lower. Related to this observation is the possible reappearance of an infection if the static drug is removed before the immune system has completely cleared the infection. Under these circumstances, the microorganism can resume growth after removal of the drug (Fig. 38-1).

According to their mechanism of cell killing, bactericidal agents can be further characterized as either **time-dependent** or **concentration-dependent** (Fig. 38-2). Time-dependent bactericidal agents exhibit a constant rate of killing that is independent of drug concentration, provided that the drug concentration is greater than the minimum bactericidal concentration (MBC). Thus, the overriding consideration for the clinical use of such agents is not the absolute drug concentration that is achieved, but for how long the drug concen-

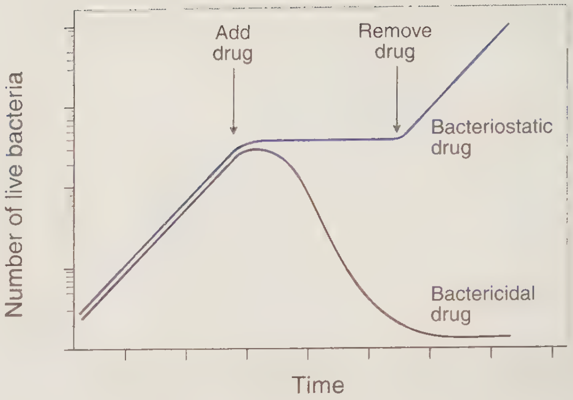


**Figure 38-1. Comparison of the Effects of Bacteriostatic and Bactericidal Drugs on Bacterial Growth Kinetics.** In the absence of drug, bacteria grow with exponential (first-order) kinetics. A bactericidal drug kills the target organism, as demonstrated by the time-dependent decrease in the number of live bacteria. A bacteriostatic drug prevents microbial growth without killing the bacteria. Removal of a bacteriostatic drug is followed by an exponential increase in bacterial number, as the previously inhibited bacteria resume growth. Bacteriostatic drugs eradicate infections by limiting the growth of the infecting organism for a long enough period of time to allow the host immune system to kill the bacteria.

**TABLE 38-1** Examples of Bactericidal and Bacteriostatic Antibiotics

| BACTERICIDAL ANTIBIOTICS | | |
|---|---|---|
| CONCENTRATION-DEPENDENT | TIME-DEPENDENT | BACTERIOSTATIC ANTIBIOTICS |
| Aminoglycosides | Beta-lactams | Chloramphenicol |
| Quinolones | Vancomycin | Clindamycin |
| Bacitracin | Polymyxins | Ethambutol |
| | Pyrazinamide | Macrolides |
| | Isoniazid | Novobiocin |
| | Rifampin | Sulfonamides |
| | Metronidazole | Tetracyclines |
| | | Trimethoprim |

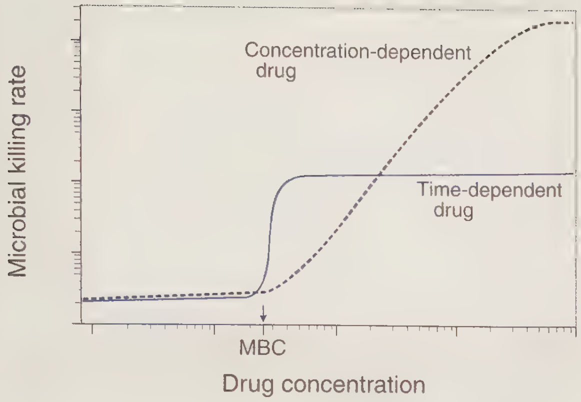


**Figure 38-2. Relationship Between Rate of Microbial Killing and Drug Concentration for Time-Dependent and Concentration-Dependent Bactericidal Drugs.** Time-dependent bactericidal agents exhibit a constant rate of microbial killing at concentrations of drug greater than the minimum bactericidal concentration (MBC) (solid line). In contrast, concentration-dependent bactericidal agents show increased killing with increasing drug concentration (dashed line). Note that the efficacy of concentration-dependent bactericidal agents eventually plateaus, because the effective concentration of the drug becomes limited by the rate of drug diffusion to its molecular target.

tration remains in the therapeutic range (which is defined as [drug] > MBC). In contrast, concentration-dependent bactericidal agents have a rate of killing that increases with drug concentration for [drug] > MBC. For such agents, a single very large dose can have a profound therapeutic effect, and may be sufficient to eliminate the infection.

## TYPES OF DRUG INTERACTIONS—SYNERGY, ADDITIVITY, AND ANTAGONISM

The discussion thus far has considered the general properties of drugs used as single agents to treat a microbial infection. When such drugs are used in combination with other agents, these effects can be modified (either enhanced or diminished). In fact, drugs that have little or no activity against an organism when used as single agents can show high activity when used in combination with another agent. One example of this concept involves the treatment of *Enterococcus fecalis,* a Gram-positive organism that exhibits little susceptibility to aminoglycosides. Recall that, according to the Davis model, aminoglycosides kill bacteria by inducing misreading of the genetic code and translation of defective proteins that cause further cellular damage (see Chapter 33). In the case of *E. fecalis,* aminoglycosides are unable to penetrate the organism's thick cell wall to reach their target—the 30S prokaryotic ribosomal subunit. However, when used in combination with a cell wall synthesis inhibitor such as vancomycin or a beta-lactam antibiotic, aminoglycosides are able to reach the bacterial ribosomes and effectively kill the cell (see Chapter 35). The potentiating effect of the cell wall synthesis inhibitor on the activity of the aminoglycoside is an example of the important pharmacologic concept of **synergy.**

From this example, one could ask whether combining two drugs with individual activity against a particular microbe always results in a more potent drug combination. Surprisingly, for many combinations, this turns out not to be the case. In fact, when two drugs with activity against the same pathogen are combined, the drugs can interact to enhance the efficacy of the combination (synergy) or to diminish the efficacy (**antagonism**). Alternatively, the drugs may not interact, and the effect of the combination is obtained by adding the effects of each drug used individually (**additivity**). The interaction between two antimicrobial drugs is often quantified by selecting a particular endpoint (e.g., inhibition of bacterial growth), and then measuring the effect of various combinations of the two drugs that produce this endpoint. When such data are plotted, additional information can be obtained (Fig. 38-3). The x- and y-intercepts correspond to the MICs of the two drugs, and the concavity of the curve indicates the nature of the interaction between the two drugs—concave-up is synergistic; concave-down is antagonistic; linear is additive. The following discussion provides a mathematical rationale for these relationships.

Suppose that drugs A and B inhibit a particular enzyme required for bacterial growth and division. In this case, the ratio $[A]/MIC_A$ would represent the fraction of bacterial growth inhibition that can be attributed to the presence of drug A. This is known as the fractional inhibitory concentration of A ($FIC_A$). Similarly, $FIC_B = [B]/MIC_B$ is the fraction of growth inhibition that can be attributed to drug B. Now suppose that the concentration of A is decreased by a

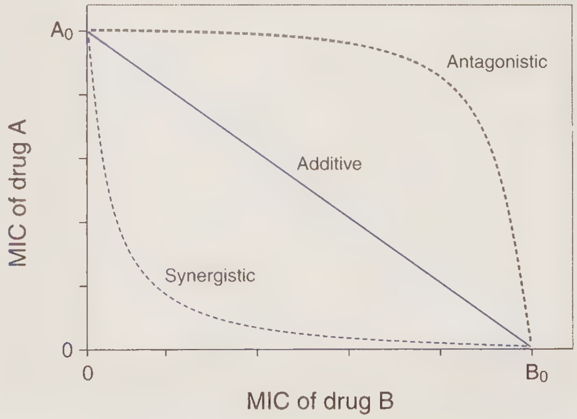


**Figure 38-3. Quantification of Additive, Synergistic, and Antagonistic Drug Interactions.** Drug combinations can exhibit additive, synergistic, or antagonistic effects. The nature of this interaction can be depicted graphically by observing the effect that each drug has on the other's minimum inhibitory concentration (MIC). If two drugs have an additive interaction, then the addition of increasing amounts of Drug B to Drug A results in a linear decrease in the MIC of Drug A; in this case, each of the two drugs can be thought of as interchangeable. If two drugs have a synergistic interaction, then the addition of Drug B to Drug A results in a significantly lower MIC for Drug A (i.e., there is an increase in the potency of Drug A). If two drugs have an antagonistic interaction, then the addition of Drug B to Drug A does not significantly lower the MIC of Drug A; in some cases (not shown), much higher doses of each drug must be administered to achieve the same effect as that of each drug used alone. $A_0$ and $B_0$ are the MICs of Drugs A and B, respectively, when used as single agents.

little bit, –d[A]. To compensate for this loss of growth inhibition ($dFIC_A = -d[A]/MIC_A$), the concentration of B must be increased by an amount +d[B].

For additive drugs, the ratio –d[A]/d[B] (which is the same as the slope of the curve in Fig. 38-3) is a constant because one unit of A has exactly the same activity as ($MIC_A/MIC_B$) units of B. For example, A and B could bind to independent sites on the enzyme and, therefore, each drug could have no effect on the binding of the other drug.

In contrast, if A and B are synergistic, then the amount of B (d[B]) required to compensate for a decrease in A (–d[A]) depends on the amount of A that is already present. Because of the potentiating effect of drug A on drug B, d[B] is less for higher [A] (i.e., $d^2[A]/d[B]^2 > 0$, which corresponds to the concave-up curve in Fig. 38-3). From a molecular perspective, this relationship could correspond to a situation in which the binding of A to the enzyme induces a conformational change in the binding site for B that enhances the binding of B.

By extension, A and B are antagonistic if the amount of B required to compensate for a small decrease in the concentration of A is larger for higher [A] (i.e., $d^2[A]/d[B]^2 < 0$, which corresponds to the concave-down curve in Fig. 38-3). For example, the binding of A could result in a lower affinity for the binding of B to the enzyme. Note that, because of its intuitiveness and simplicity, the mathematical model described above is often used to define synergy, additivity, and antagonism. It is not, however, the most general formulation of the quantitative analysis of the effects of multiple drugs, which is beyond the scope of this text. The interested reader is referred to the work of Chou and Talalay (1984) for more detailed coverage of this subject.

Several generalizations can be made concerning the nature of drug interactions between different classes of antimicrobial agents. First, many bacteriostatic drugs (e.g., tetracycline, erythromycin, chloramphenicol) antagonize the action of bactericidal drugs (e.g., vancomycin, penicillin) by inhibiting cell growth and/or preventing the cellular processes that are required for cidal drugs to act. Second, two bactericidal drugs usually act synergistically in combination. One notable exception to the latter generalization is that rifampin, a bactericidal inhibitor of RNA polymerase, antagonizes other bactericidal drugs by inhibiting cell growth. Finally, the interactions between two bacteriostatic drugs are often additive, but cannot be predicted in all cases.

## EXAMPLES OF ANTIMICROBIAL COMBINATION THERAPY

There are several compelling reasons for using combinations of drugs to treat microbial infections, including: (1) to prevent the emergence of resistance; (2) to enhance the activity (efficacy) of the drug therapy against a specific infection (synergy); (3) to reduce toxicity to the host; (4) to treat multiple simultaneous infections (sometimes called polymicrobial infections); and (5) to treat a life-threatening infection empirically before the microorganism causing the infection has been identified.

### Tuberculosis

The treatment of tuberculosis illustrates one of the principal reasons combinations of drugs are used—to suppress the emergence of resistance. In the course of this illness, tuberculous bacilli are inhaled and phagocytosed by alveolar macrophages, where the bacilli multiply within intracellular vacuoles. A predominantly T-cell-mediated lymphocytic response is then elicited, and the macrophages and helper T cells form large granulomas that wall off the infected sites. Activated macrophages are usually able to keep the infection under control by killing the multiplying bacilli, but are unfortunately unable to eradicate the infection completely. Tissue damage is caused by the release of neutral proteases and reactive oxygen intermediates from activated macrophages, with the end result that central necrosis occurs in the tuberculous cavities in the lungs. Inside each of these cavities, as many as $10^8$–$10^9$ living bacilli may be held in check by macrophages and helper T cells.

Successful treatment of tuberculosis infections typically requires the use of combinations of drugs with antimycobacterial activity. Commonly used drugs include isoniazid, rifampin, ethambutol, pyrazinamide, and streptomycin (see Chapter 35). A standard regimen could consist of 2 months of isoniazid, rifampin, and pyrazinamide, followed by 4 months of isoniazid and rifampin. Ethambutol and streptomycin are sometimes substituted for one or two drugs in this regimen if resistance develops. Isoniazid and rifampin are the preferred drugs because of their ability to kill intracellular as well as extracellular mycobacteria. The other drugs are either bacteriostatic (ethambutol), ineffective against intracellular bacilli (streptomycin), or hepatotoxic (pyrazinamide). Resistance to these drugs develops primarily through mutations, and the frequency of resistance to any one of the drugs is about 3 per $10^7$ bacilli. Considering that a tuberculous cavity contains as many as $10^8$–$10^9$ bacteria, it is not surprising that treatment with one drug alone results in selection for bacilli that are resistant. For example, resistance to isoniazid, an inhibitor of mycolic acid synthesis, usually occurs by a mutation in the gene that codes for fatty acid synthetase 2 (see Chapter 35). In fact, a brief 3 weeks of treatment with isoniazid as a single agent can result in a frequency of resistance of 40% in clinical isolates.

When two antimycobacterial drugs are combined, however, the frequency of resistance to both drugs is 3 in $10^7$ times 3 in $10^7$, or ~1 in $10^{13}$, which is much lower than the number of bacilli present in the infection. Under these circumstances, bacteria that are resistant to one drug remain susceptible to the other. Furthermore, because the drugs used in this combination have distinct molecular targets, resistance to one drug does not confer resistance to the other.

### Synergistic Combinations

A second reason for using a combination drug regimen is to take advantage of the synergy between the actions of the two drugs. This becomes increasingly important in the setting of infections that are not readily handled by the immune defenses of immunocompromised patients. In the immunocompetent patient, bacteriostatic and bactericidal drugs are often equally efficacious in eliminating an infection. Bactericidal drugs are strongly preferred, however, in the settings of immunocompromised patients (e.g., HIV/AIDS patients, immunosuppressed transplant patients, and neutropenic cancer patients), endovascular infection (e.g., bacterial endocarditis), or meningitis. The reason for using bactericidal combinations in the immunocompromised patient should be obvious—the host does not have sufficient numbers of functioning lymphocytes and/or neutrophils to eliminate even a nondividing bacterial population. In the case of endocarditis, the reason is not so straightforward. In this case, although there is not a deficiency in the absolute number of leukocytes, the phagocytes are unable to penetrate efficiently the thick "vegetation"—composed of a meshwork of fibrin, platelets, and bacterial products—that surrounds the bacteria. Bactericidal drug combinations are often indicated for meningitis to maximize the probability of overcoming the poor opsonization of bacteria by antibody and complement in the immunologically privileged site of the meninges (see Chapter 6, Principles of Nervous System Physiology and Pharmacology).

One example of antibacterial synergy involves the use of a penicillin and an aminoglycoside to treat the most common causes of acute and subacute bacterial endocarditis, *Staphylococcus aureus* and *Streptococcus viridans,* respectively. As described above, the mechanism of synergy relies on the penicillin inhibiting cell wall biosynthesis, which allows the aminoglycoside to penetrate the thick peptidoglycan layer of these Gram-positive organisms.

Two other commonly used synergistic combinations include either amphotericin B and flucytosine, or a sulfonamide and trimethoprim or pyrimethamine in fungal infections. These classic examples serve to illustrate two basic mechanisms whereby one drug can potentiate the activity of another. Analogous to the action of penicillins, which enhance the uptake of aminoglycosides by Gram-positive bacteria, amphotericin B enhances flucytosine uptake by fungal cells by damaging ergosterol-rich fungal cell membranes (see Chapter 36, Pharmacology of Fungal Infections). Only after penetrating the fungal membrane can flucytosine be converted into its active form (5-fluorouracil, which is converted to 5-FdUMP, an irreversible inhibitor of thymidylate synthase) by a fungal-specific deaminase. Because of the particularly low therapeutic index of amphotericin B (which is primarily a consequence of its nephrotoxicity), this combination has the great advantage of reducing the dose of amphotericin B required to treat a systemic fungal infection such as cryptococcal meningitis.

Sulfamethoxazole and trimethoprim are commonly used in combination in the treatment of *Pneumocystis carinii* pneumonia, an opportunistic infection frequently encountered in patients with AIDS, as well as many urinary tract infections caused by Gram-negative enterics. An analogous combination, sulfadoxine and pyrimethamine, is used in the treatment of malaria, toxoplasmosis, and other protozoal infections. These combinations illustrate a second mechanism whereby drugs can exert a synergistic effect. The mechanism of synergy is based on the inhibition of two steps in the folic acid biosynthesis pathway that each affects the cellular concentration of the same critical metabolite, dihydrofolate (see Chapter 31, Pharmacology of Nucleotide Synthesis). The reduced form of this metabolite, tetrahydrofolate, is a required substrate for purine biosynthesis and for many one-carbon transfer reactions, and is, thus, necessary for DNA replication and cell division (see Fig. 31-6).

The sulfonamides are competitive inhibitors of dihydropteroate synthase, the enzyme that catalyzes the first step in the synthesis of tetrahydrofolate from PABA and pteridine. Trimethoprim and pyrimethamine inhibit a subsequent step in this pathway, acting as competitive inhibitors of the bacterial and protozoal isoforms of dihydrofolate reductase (DHFR), respectively. The sulfonamide-induced reduction in the cellular concentration of dihydrofolate acts synergistically with trimethoprim or pyrimethamine, because the latter drugs compete with dihydrofolate for binding to DHFR. (In other words, the action of trimethoprim or pyrimethamine is enhanced because the sulfonamide acts to decrease the concentration of dihydrofolate, the substrate that competes with these drugs for binding to the enzyme.) Moreover, resistance to this combination cannot develop easily because strains that are resistant to trimethoprim usually possess an altered DHFR that has a lower affinity for dihydrofolate. Under these circumstances, the lower concentration of dihydrofolate that results from the action of the sulfonamide is insufficient to allow the altered DHFR to meet the cellular needs for tetrahydrofolate. For resistance to develop to the drug combination, the cell would simultaneously have to overproduce PABA (to overcome the competitive inhibition by the sulfonamide) and mutate its DHFR (to decrease the affinity of this enzyme for trimethoprim). This combination of events is unlikely to occur in a single bacterial or protozoal cell.

## Coadministration of Penicillins with β-Lactamase Inhibitors

The combination of a β-lactam antibiotic and a β-lactamase inhibitor (e.g., clavulanic acid, sulbactam, tazobactam) illustrates a mechanism of drug interaction that is not technically synergistic (because the β-lactamase inhibitor has no antibacterial activity of its own) but that shares a functional similarity with the drug combinations discussed above. Clavulanic acid is an inhibitor of β-lactamase, an enzyme used by many Gram-positive and Gram-negative bacteria to inactivate penicillins (see Chapter 35). By preventing the hydrolysis and inactivation of penicillins, clavulanic acid (and other β-lactamase inhibitors) greatly increases the potency of penicillins (and other β-lactams) against bacteria that express β-lactamase. This combination has been effective in the treatment of infections due to penicillin-resistant *S. pneumoniae,* which is a common cause of otitis media in infants. Such organisms have typically acquired resistance to penicillins through a plasmid-encoded beta-lactamase.

## Polymicrobial and Life-Threatening Infections

Combinations of antimicrobial drugs are used not only to prevent the emergence of resistance and to act synergistically against a specific, known pathogen, but also to treat polymicrobial infections and infections in which treatment must be initiated before the identification of the microbe causing the infection. Consider, for example, the case of a ruptured appendix or colonic diverticulum that has leaked bacteria into the peritoneal cavity. Such an intra-abdominal abscess is likely to contain a wide spectrum of microorganisms—much too broad to be targeted effectively by a single antibiotic. After draining the abscess, treatment with a combination of antibacterial agents such as an aminoglycoside—to kill aerobic Gram-negative Enterobacteriaceae (e.g., *E. coli*)—and clindamycin or metronidazole—to kill anaerobes (e.g., *Bacteroides fragilis*)—often results in clearance of the infection. In cases where presumptive treatment is indicated before the causative microorganism is identified, body fluids such as blood, sputum, urine, and CSF should be cultured before initiating therapy. A combination of drugs with activity against the microbes that are most likely to be involved in the infection (or that could result in the most serious outcome) is then administered until a positive bacteriologic identification is made and drug susceptibility results are obtained. At that point, it may be possible to discontinue unnecessary drugs and to implement specific and potent monotherapy.

## UNFAVORABLE DRUG COMBINATIONS

Antagonism can sometimes result from combination chemotherapy, although this situation is to be avoided if possible. Antagonism is most commonly observed when static drugs are used in combination with cidal drugs. For example, tetracyclines are bacteriostatic antimicrobials that antagonize the bactericidal activity of penicillins (see Chapter 33). Recall that the bactericidal activity of penicillins depends on cell growth. By inhibiting the transpeptidation reaction involved in bacterial cell wall crosslinking, the penicillins create an imbalance between cell wall synthesis and autolysin-mediated cell wall degradation. If the bacterial cell continues to grow, this leads to spheroplast formation and, eventually, to osmotic lysis. A protein synthesis inhibitor such as tetracycline, which arrests cell growth, would therefore antagonize the effect of a β-lactam. Similarly, imidazoles and triazoles are fungistatic agents that antagonize the fungicidal activity of amphotericin B (see Chapter 36). The mechanism of antagonism can be appreciated by noting that amphotericin B acts by binding ergosterol and forming pores in the fungal membrane, whereas imidazoles and triazoles inhibit a microsomal cytochrome P450-dependent enzyme, 14α-sterol demethylase, that is involved in ergosterol biosynthesis. Thus, the imidazoles and triazoles oppose the action of amphotericin B by decreasing the concentration of the target for the latter drug. (Note that static and cidal antimicrobial drugs are sometimes used clinically in combination when no good alternatives exist. In such cases, it may be required to increase the dose of the static and/or cidal drug to overcome the antagonistic drug–drug interaction. This can lead to an increased probability of toxic side effects caused by elevated levels of one or both of the drugs in the combination.)

## ANTIVIRAL COMBINATION THERAPY: HIV

Soon after zidovudine (AZT) was approved for use in the treatment of human immunodeficiency virus (HIV) infection, it was appreciated that this monotherapy had only a modest effect in suppressing viral replication. Treatment with single-agent AZT was shown to reduce initial viral load by approximately 3- to 10-fold, but drug resistance almost inevitably occurred after 6 months to 3 years of treatment. Many anti-HIV drugs have been introduced since the advent of AZT (see Chapter 34, Pharmacology of Viral Replication), including other nucleoside analogue reverse transcriptase (RT) inhibitors (e.g., 3TC), non-nucleoside RT inhibitors (NNRTIs, e.g., nevirapine), and protease inhibitors (e.g., indinavir). However, although the newer drugs have shown more dramatic effects in reducing initial viral load (e.g., protease inhibitors usually cause a > 30-fold decrease in viral RNA levels), all of these drugs have failed to show long-term suppressive benefit when used individually. In fact, resistance to lamivudine (3TC), NNRTIs, and protease inhibitors develops more quickly than does resistance to AZT.

The viral life cycle is central to understanding the reason that monotherapy for HIV fails to suppress long-term viral replication (see Chapter 34, Fig. 34-2). After virus binding and fusion, the viral enzyme RT synthesizes double-stranded DNA from the single-stranded viral RNA genome. The DNA is then integrated into the host chromosome and transcribed over and over using the host cell's transcription machinery. These complete genomic transcripts are used to make viral capsid proteins, envelope proteins, RT, integrase, and protease, which are assembled into immature virions that bud from the plasma membrane. The immature virions undergo a series of proteolytic cleavage events to gain infectivity.

Efforts to interrupt this life cycle have focused primarily on inhibiting the early reverse transcription and late protease maturation steps. However, HIV RT is relatively unfaithful, so replication error rates are quite high (about 1/1000 bp). In addition, transcription of the integrated DNA into RNA is also error prone. Although the resulting error rate is not so high as to be intolerable to the virus, it is sufficiently high that, after repeated cycles of infection, reverse transcription, and transcription, a significant number of viruses encode altered targets of anti-HIV therapy and thereby acquire resistance, even prior to treatment.

In the setting of high mutation rates, combination chemotherapy is beneficial. Combinations of RT inhibitors (e.g., AZT and 3TC) are more effective than one RT inhibitor alone, in part because resistance to one nucleoside analogue does not necessarily confer resistance to another. The current standard of care for treatment of HIV infection is "triple therapy." Triple therapy can use a nucleoside analogue RT inhibitor in combination with an NNRTI and a protease inhibitor, or two nucleoside analogues and a protease inhibitor, or even three different RT inhibitors. Clinical trials have shown that such combinations are able to reduce viral RNA plasma levels below the limit of detection (currently, 20–200 copies/mL). At such low levels of viral replication, the probability of resistance emerging to any one of the drugs is greatly reduced. Thus, for example, it has been shown that combinations including NNRTIs and protease inhibitors remain effective for much longer periods of time than does either class of drugs administered as a single agent. However, the complicated administration schedules and side effects of such combinations can reduce compliance. Thus, although some investigators are optimistic that early aggressive treatment with combinations of drugs can suppress viral replication indefinitely, others prefer to wait before initiating such aggressive treatment.

## ANTINEOPLASTIC COMBINATION CHEMOTHERAPY

Antineoplastic chemotherapy faces a number of intrinsic difficulties. *Cancer cells can be thought of as "altered self" cells that maintain a number of similarities to normal, noncancerous cells, making it difficult to target the cancer cells* *specifically*. Also, the currently available cancer chemotherapeutic agents have numerous side effects that often limit their dose and frequency of administration. Despite these hurdles, combination chemotherapy has led to remarkable advances in the treatment of cancer, including the examples of Hodgkin's disease and testicular cancer discussed at the end of this section. Table 38-2 provides an overview of the major antineoplastic drug classes, including their mechanisms of action, cell cycle specificities, major resistance mechanisms, and dose-limiting toxicities. Note that all of these drug classes have been discussed in previous chapters; the following discussion integrates relevant information about the individual drugs in a clinical context.

## GENERAL CONSIDERATIONS

It has been said that cancer is a disorder of the cell cycle. To appreciate the challenges that must be faced in treating cancer with drug therapies, it is useful to examine the current model for oncogenic transformation. Normal somatic cells undergo differentiation as they mature from a small regenerating stem cell population. Because cells lose the ability to divide as they progress further along their differentiation pathway, it is not surprising that malignancies tend to arise in populations of immature or undifferentiated cells. At the molecular level, the process of malignant transformation involves multiple steps, including the loss of tumor suppressor gene products (e.g., p53 and Rb) and the activation of proto-oncogenes (e.g., ras and c-myc) through such processes as somatic mutation, DNA translocation, and gene amplification. Acquired alterations in genes that regulate the progression of cells through the cell cycle confer a growth advantage on malignant cells, which proliferate in the absence of normal growth regulatory signals. Some of the most aggressive transformed cells multiply at a rate of about two divisions per day. At this rate, a single such cell could give rise to a clinically detectable mass of 1 gram ($10^9$ cells) in just 15 days, and a tumor burden of 1 kilogram ($10^{12}$ cells), which is often incompatible with life, could be achieved in 20 days.

Fortunately, oncogenesis usually occurs much more slowly than this—a fact that supports the concept of screening for many types of cancers (e.g., cervical, prostate, and colon). A malignant cell can give rise to a small colony of cells ($10^6$ cells) rather quickly, but further growth is held in check by the limited availability of oxygen and nutrients. Because oxygen can diffuse passively in tissues over a distance of only 2–3 mm, cells in the center of the growing tumor mass become hypoxic and enter the $G_0$ (resting) phase. Accordingly, the percentage of cells that are actively dividing (i.e., the growth fraction of the tumor) decreases as tumor size increases. Moreover, the continued proliferation of cells at the tumor margins causes a further decrease in the $pO_2$ in the center of the tumor, and hypoxic tumor cells begin to die (central necrosis). The tumor continues to grow, albeit at a slower rate, because the rate of cell division at the margins exceeds the rate of central necrosis. At some point, hypoxic tumor cells can express or induce the stromal expression of angiogenic factors (e.g., vascular endothelial growth factor, VEGF) that induce vascularization of the tumor. Vascularization can be accompanied by a sudden increase in the growth fraction, as cells are pulled out of $G_0$ phase and into the cell cycle.

Because a single malignant cell can expand clonally to give rise to a tumor, it is thought that every malignant cell must be destroyed to effect a cure of the cancer. This hypothesis, together with the "log-kill" hypothesis for tumor cell killing (see Chapter 30), suggests that multiple cycles of chemotherapy must be administered at the highest tolerable doses and the most frequent tolerable intervals to achieve a cure. Antineoplastic chemotherapy usually follows first-order kinetics (i.e., a constant *fraction* of tumor cells is killed with each cycle of chemotherapy). These kinetics of tumor cell killing are unlike the time-dependent killing characteristic of many antimicrobial drugs, which follows zero-order kinetics (i.e., a fixed *number* of microbes is killed per unit time).

Adding to the difficulty of successful cancer treatment is the phenomenon of tumor progression, in which a clonally derived population of malignant cells becomes heterogeneous through the accumulation of multiple genetic alterations (mutations). When subjected to selective pressure by immune surveillance or the administration of an antineoplastic agent, subclones of the tumor with relatively nonantigenic or drug-resistant phenotypes are selected for in Darwinian fashion. Mutations that confer drug resistance are of particular concern, because many transformed cells, having lost the ability to repair DNA damage, are characterized by genomic instability. Thus, deletions, gene amplifications, translocations, and point mutations are not infrequent events, and can result in antineoplastic drug resistance through any of the mechanisms shown in Table 38-3.

With the possible exception of several recently developed classes of therapies that are based on molecular targets selectively expressed by a malignant clone of cells (e.g., a monoclonal antibody directed against a tumor cell antigen—see Chapter 49, Protein-Based and Gene Therapies—or an enzyme inhibitor directed against a mutated signal transduction molecule—see Chapter 1, Drug-Receptor Interactions), antineoplastic chemotherapy has focused on disrupting the cell cycle in rapidly dividing cells. Some of these agents act by inducing DNA damage and subsequent apoptosis at all phases of the cell cycle, whereas others act selectively in one phase of the cell cycle (see Chapter 30, especially Fig. 30-4). Unfortunately, such drugs are also associated with significant host toxicity, especially in tissues that normally have a high rate of cell turnover (e.g., bone marrow, hair follicles, intestinal epithelia). Accordingly, neutropenia, thrombocytopenia, anemia, alopecia, nausea, and oral and intestinal ulcerations are common side effects of many antineoplastic agents.

Although many rapidly growing lymphomas and leukemias seem to melt away with antineoplastic chemotherapy, more indolent solid tumors must be treated with adjuvant (i.e., chemotherapy-enhancing) radiation therapy and/or surgery. By the time these tumors come to clinical attention, they are often quite large and may have metastasized widely. In such cases, removal of the primary tumor is often followed by radiation therapy and/or systemic chemotherapy, using agents that penetrate various tissues (e.g., brain, liver) that could be involved with metastatic disease.

In summary, cancer therapy must eliminate every malignant cell from the body, making high doses of chemotherapeutic agents desirable. (In practice, immune mechanisms may be able to clear small numbers of remaining cancer cells, if these cells are sufficiently immunogenic.) However, the toxicity of these relatively nonselective agents limits their

## TABLE 38-2 Classes of Cancer Chemotherapeutic Agents

| DRUG CLASS | MECHANISM OF ACTION | CELL CYCLE SPECIFICITY | MAJOR RESISTANCE MECHANISM | DOSE-LIMITING TOXICITY |
|---|---|---|---|---|
| Alkylating agents | Crosslink DNA, RNA, protein | Nonspecific | ↑ DNA repair, ↓ drug uptake, ↑ drug inactivation | Bone marrow |
| Antimetabolites | Disrupt nucleotide synthesis, utilization, incorporation | S | ↓ drug uptake, ↓ drug activation, ↑ drug inactivation, ↑ or altered target enzyme, salvage pathway | Bone marrow |
| Natural products | | | | |
| Vinca alkaloids | Disrupt microtubule assembly | M | ↑ drug efflux | |
| Taxanes | Disrupt microtubule disassembly | M | ↑ drug efflux | Bone marrow / neuropathy |
| Epipodophyllotoxins | Inhibit topoisomerase II | S / G2 | ↑ drug efflux | Bone marrow (mild) |
| Camptothecins | Inhibit topoisomerase I | S | ↑ drug efflux? | Bone marrow / diarrhea |
| Anthracyclines | DNA intercalation, inhibit topoisomerase II, lipid peroxidation | G2 | ↑ drug efflux | Bone marrow / heart |
| Bleomycin | DNA strand scission | G2 | ↑ drug inactivation? | Pulmonary fibrosis |
| Miscellaneous | | | | |
| Platinum complexes | DNA intrastrand crosslinks (G-G) | Nonspecific | ↑ DNA repair, ↓ drug uptake, ↑ drug inactivation | Renal |
| Hydroxyurea | Inhibits ribonucleotide reductase | S | | Bone marrow |
| Monoclonal antibodies | Target tumor-specific antigen | | | |
| Toxin conjugates | Target tumor-specific antigen | | | |
| Hormones / antagonists | | | | |
| Prednisone | Glucocorticoid receptor agonist | G1 | Loss of hormone sensitivity (↑ or altered target receptor) | Cushingoid syndrome |
| Tamoxifen | Estrogen receptor antagonist | | Loss of receptor-dependent growth | |
| Anastrozole | Aromatase inhibitor | | | |
| Methyltestosterone | Androgen receptor agonist | | | |
| Flutamide | Androgen receptor antagonist | | | |
| Leuprolide | GnRH receptor "superagonist" | | | |
| Biological response modifiers | | | | |
| Interferon-alpha | Interferon receptor agonist | | | Bone marrow / neurotoxic / heart |
| Interleukin-2 | IL-2 receptor agonist (proliferation, differentiation of T cells) | | | Hypotension / pulmonary edema |
| Differentiating agents | Induce differentiation of cancer cells | | | |
| Tretinoin | Retinoic acid α receptor agonist | | | |

**TABLE 38-3** Mechanisms of Tumor Resistance to Chemotherapeutic Agents

| MECHANISM OF TUMOR RESISTANCE | EXAMPLES |
|---|---|
| Insufficient drug uptake | Methotrexate, doxorubicin |
| Increased inactivation of drug | |
| Cytidine deaminase overexpression | Ara-C |
| Alkaline phosphatase overexpression | 6-TG, 6-MP |
| Insufficient activation of drug | 5-FU, 6-MP, Ara-C, 6-TG |
| Overexpression or alteration of target enzyme | |
| Dihydrofolate reductase | Methotrexate |
| Increased utilization of alternate pathways | Antimetabolites |
| Repair of drug-induced lesions | Alkylating agents |
| Increased concentration of competing metabolites (dCTP) | Ara-C |
| Efflux of drug from tumor cell (MDR phenotype) | Vinca alkaloids, etoposides, doxorubicin |
| Resistance to apoptosis | Most antineoplastics |

achievable doses. Moreover, resistance to these drugs can develop through genetic alterations. Finally, because these agents target mainly rapidly dividing cells, antineoplastic drugs are much less effective against large solid tumors with low growth fractions. Each of these considerations points to the need for combination drug regimens to treat cancer. The basic pharmacologic principles for such regimens are discussed below.

## RATIONALE FOR COMBINATION CHEMOTHERAPY

In antineoplastic chemotherapy, combination drug regimens typically include agents that act on different molecular targets, at different phases of the cell cycle, and with different dose-limiting toxicities (Table 38-2). This strategy targets asynchronously dividing tumor cells, reduces the emergence of drug resistance, and allows each drug to be given at its highest tolerable dose, thereby maximizing efficacy without excessive toxicity. Recent advances in supportive therapy have also increased the maximum tolerated doses for many antineoplastic agents. For example, the routine use of antiemetics, autologous bone marrow transplantation, hematopoietic growth factors (e.g., GM-CSF, G-CSF, IL-3, erythropoietin), and prophylactic broad-spectrum antibiotics has reduced the complications of myelosuppressive chemotherapy regimens. Similarly, allopurinol treatment to prevent the hyperuricemia that could result from widespread release and metabolism of purines from necrotic tumor cells (i.e., tumor lysis syndrome) has reduced morbidity associated with high doses of systemic chemotherapy (see Chapter 46, Integrative Inflammation Pharmacology: Gout). Finally, so-called "leukovorin rescue" after high-dose methotrexate administration selectively spares nonmalignant cells from death associated with tetrahydrofolate depletion (see Chapter 31).

Unlike the treatment of bacterial and viral infections, chemotherapy for cancer often employs an intermittent dosing strategy. The main rationale for this strategy is to avoid unacceptable toxicity to normal cells and tissues, for example, by allowing time for bone marrow recovery. Intermittent dosing may also have the advantage of "pulling" some nondividing cells out of $G_0$ and making them more susceptible to subsequent cycles of chemotherapy. The latter rationale has prompted the use of adjuvant radiation therapy and the inclusion of cell cycle nonspecific drugs in certain combination chemotherapy regimens; both of these strategies have been noted to increase significantly the growth fractions of tumors in some studies. Despite these considerations, continuous delivery of chemotherapeutic agents is occasionally beneficial in treating slowly cycling tumors (e.g., multiple myeloma) or in cases where bolus infusion of drug is associated with significantly higher toxicity (e.g., anthracyclines).

Finally, some antineoplastic drug combinations take advantage of known synergisms. A clinically important example of this is the interaction between 5-FU and methotrexate. These drugs are used in combination in the treatment of many adenocarcinomas, including breast, colon, and prostate cancers. Both drugs are S-phase specific and have common dose-limiting toxicities (bone marrow and intestinal mucosal damage), so their use in combination may seem surprising at first glance (see Chapter 31). The mechanism of synergy seems to be the enhanced activation of 5-FU in the presence of methotrexate. Recall that 5-FU is metabolized by cellular salvage pathways that ultimately convert the drug into the active form 5-FdUMP, which irreversibly inhibits the enzyme thymidylate synthase. The first step in the activation of 5-FU is catalyzed by the enzyme phosphoribosyl transferase: 5-FU + PRPP $\rightarrow$ 5-FUMP + $PP_i$. Methotrexate, an inhibitor of purine biosynthesis, enhances these salvage pathways. In particular, cells treated with methotrexate have elevated levels of 5-phosphoribosyl-1-pyrophosphate (PRPP), which favors the conversion of 5-FU to 5-FUMP and, ultimately, to 5-FdUMP by the action of ribonucleotide reductase.

## EXAMPLES OF ANTINEOPLASTIC COMBINATION CHEMOTHERAPY

### Hodgkin's Disease

The treatment of Hodgkin's disease (HD) illustrates the rational use of antineoplastic drug combinations. In this disease, there is clonal proliferation of Reed–Sternberg (RS) cells within a dense, reactive inflammatory cell background. HD originates in a single lymph node, and progresses in a contiguous fashion involving adjacent lymphoid tissues. The RS cell is the neoplastic cell; this cell seems to be of B-cell origin, making the disease a true lymphoma. Pathologic subtypes, defined on the basis of RS cell morphology and the pattern of surrounding reactive inflammatory changes, include nodular sclerosing, mixed cellularity, and lymphocyte-depleted HD.

Patients typically present with lymphadenopathy (cervical, supraclavicular, axillary, or inguinal) and/or systemic symptoms including fever, malaise, pruritus, night sweats, and weight loss. The stage of the disease determines treatment; patients with early-stage disease (stages I and II) receive radiation with or without chemotherapy, and patients with advanced-stage disease (stages III or IV) require combination chemotherapy (Table 38-4).

Before the introduction of alkylating agents in the mid-1960s, single agent chemotherapy for advanced HD resulted in a median survival of 1 year. With the development of MOPP (mechlorethamine, vincristine, procarbazine, and prednisone), the first successful antineoplastic drug combination, half of these patents were cured of their disease. Treatment remained limited by significant toxicity, however, including early gastrointestinal and neurological complications as well as late sterility and secondary malignancies (myelodysplastic syndrome, acute nonlymphocytic leukemia, and non-Hodgkin's lymphoma). Further investigation led to the development of the ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) combination, which is often less toxic and at least as effective as MOPP. ABVD or an ABVD/MOPP combination remains the current standard of care for advanced stage HD. The rationale for the ABVD drug combination comes from the knowledge that it combines both cell cycle selective and nonselective agents as well as drugs with different dose-limiting toxicities. Compared to MOPP, ABVD is associated with significantly fewer hematological and gonadal complications and secondary malignancies.

### Testicular Cancer

The principles of antineoplastic combination chemotherapy are also exemplified in the treatment of testicular cancer. This tumor arises from the spermatogenic epithelium of the testis, and is usually detected as a testicular mass on physical examination. The tumor metastasizes through lymphatic channels to pelvic and periaortic lymph nodes before disseminating widely through hematogenous routes. Treatment of local disease (without evidence of metastasis) involves surgical removal of the affected testis with or without pelvic radiation. Advanced disease requires systemic treatment with combination chemotherapy. The standard of care is PVB (Fig. 38-4). Of the three drugs used in this regimen (cisplatin, vinblastine, and bleomycin), cisplatin is the cell cycle nonspecific drug that may draw nondividing tumor cells into the actively cycling pool, where they are susceptible to the action of the cell cycle specific agents bleomycin and vinblastine. The drugs in this combination have different molecular targets, act on different phases of the cell cycle, and have different dose-limiting toxicities. Intermittent dosing allows each affected organ system (pulmonary, renal, and bone marrow) time to recover between cycles. After surgical removal of the primary tumor, such a regimen usually results in a cure.

## TREATMENT OF REFRACTORY OR RECURRENT DISEASE

Although combination chemotherapy has resulted in vastly improved survival for some cancers, many cancers become refractory to standard combination chemotherapy. If a standard chemotherapy regimen fails, other options include experimental drug therapies, palliative care, or novel drugs approved for use after treatment failure. Many patients choose to enroll in experimental clinical trials. This decision may be based on the hope that an investigational agent could prove efficacious, but with the understanding that the true benefit may be realized only by future patients. Palliative and hospice care are alternatives to continued drug treatment in cases of advanced metastic disease. An increasing number of agents

**TABLE 38-4** The Ann Arbor Staging System for Hodgkin's Disease

| STAGE | DESCRIPTION | MODIFIERS |
|---|---|---|
| I | Involvement of a single lymph node region | A: No systemic symptoms<br>B: Systemic symptoms (e.g., fever, night sweats, weight loss)<br>E: Extranodal contiguous extension |
| II | Involvement of two or more lymph node regions on the same side of the diaphragm | A: No systemic symptoms<br>B: Systemic symptoms<br>E: Extranodal contiguous extension |
| III | Involvement of nodal regions on both sides of the diaphragm | A No systemic symptoms<br>B: Systemic symptoms<br>S: Splenic involvement<br>E: Extranodal contiguous extension |
| IV | Disseminated disease involving multiple extralymphatic organs (e.g., liver, spleen, bone marrow) | A: No systemic symptoms<br>B: Systemic symptoms |

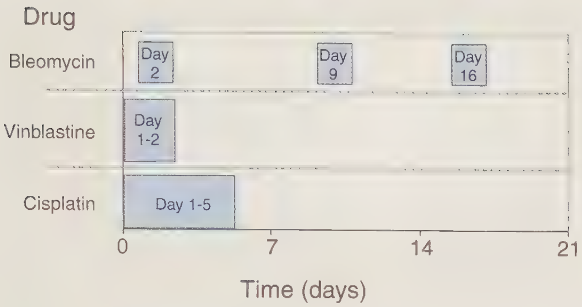


**Figure 38-4. The Platinum-Vinblastine-Bleomycin (PVB) Combination Chemotherapy Regimen for Testicular Cancer.** The PVB regimen used to treat testicular cancer consists of a combination of cisplatin, vinblastine, and bleomycin. Cisplatin is cell cycle nonspecific; this drug may draw nondividing cells into the cell cycle, where they can be killed by the G2 phase-specific agent bleomycin and the M phase-specific agent vinblastine. The intermittent dosing schedule limits drug toxicity and allows time for the bone marrow to recover from drug-induced myelosuppression. The 3-week cycle shown here is typically administered four times in succession (12 weeks total).

with novel mechanisms of action, however, are becoming available for disease that is otherwise treatment-refractory. Two recently approved classes of agents include proteasome inhibitors and anti-CD20 monoclonal antibodies.

The proteasome functions to degrade cellular proteins that have been targeted for destruction by addition of a polyubiquitin tag. **Bortezomib** selectively inhibits proteasome activity, apparently by competitively interfering with the protease activity of the proteasome 26S subunit. Inhibition of the proteasome may result in decrease activity of nuclear factor-κB (NF-κB), a signaling protein that has been implicated in many cancers. The selectivity of bortezomib for neoplastic cells is unclear, but studies have shown that bortezomib inhibits growth and induces apoptosis in many neoplastic cell types, including multiple myeloma. Bortezomib has been approved for use in multiple myeloma that has progressed despite at least two previous treatment regimens. The major side effects consist of peripheral neuropathy, gastrointestinal distress, and thrombocytopenia. Ongoing clinical trials are investigating the potential utility of bortezomib in other malignancies.

An increasing number of novel therapies consist of monoclonal antibodies directed against a cell surface target unique to the cell of interest (see Chapter 43, Pharmacology of Immunosuppression, and Chapter 49, Protein-Based and Gene Therapies). Binding of the antibody to the target cell can cause selective cell depletion by subsequent activation of complement, induction of apoptosis, or local delivery of a toxic agent (e.g., radiation or a toxin). In the case of leukemias and lymphomas, this approach involves targeting of immune cell antigens expressed preferentially on neoplastic cells.

A subset of Non-Hodgkin's lymphomas (NHLs) includes a heterogenous group of indolent tumors that respond increasingly poorly to continued rounds of chemotherapy. Approximately 85% of these lymphomas are B cell-derived tumors that selectively express CD20, a cell-surface antigen normally expressed during B cell development. **Rituximab** is an anti-CD20 antibody used in the treatment of NHL. Although more than 50% of patients initially experience remission (defined as 6 months of disease-free survival), most patients eventually relapse after rituximab therapy. An alternative approach employing radiolabeled anti-CD20 antibodies has recently been approved for use in recurrent or refractory CD20$^+$ NHL. **Ibritumomab tiuxetan** (marketed under the trade name Zevalin) is a $^{90}$Y-conjugated anti-CD20 monoclonal antibody. **Tositumomab** (marketed under the trade name Bexxar) is a $^{131}$I-conjugated anti-CD20 monoclonal antibody. Ibritumomab is approved for use in recurrent or refractory NHL, while tositumomab is approved for use only in recurrent or refractory NHL that is also refractory to rituximab. The initial results with these radiolabeled antibodies have been promising. Ibritumomab therapy results in remission rates of approximately 80%, with more than 25% of patients achieving complete remission. Tositumomab therapy results in remission rates of approximately 95%, with more than 60% of patients achieving complete remission. Both agents require complicated dosing strategies and treatment regimens, and should be administered only by physicians with specialized experience with the agents.

## *Conclusion and Future Directions*

The principles of combination chemotherapy discussed in this chapter highlight the importance of combination drug treatment in a variety of clinical situations. The use of drug combinations has greatly enhanced the effectiveness of treatment of both infectious and neoplastic diseases. The advantages offered by multidrug regimens over individual drug therapy (monotherapy) include increased antimicrobial, antiviral, and antineoplastic efficacy, decreased overall drug resistance, decreased host toxicity, and broader coverage of suspected pathogenic organisms. These advantages are illustrated in the rational use of drug combinations to treat infections with *Mycobacterium tuberculosis* and HIV, as well as such neoplastic disorders as Hodgkin's disease and testicular cancer. Continued refinement of combination chemotherapy regimens will rely on increased understanding of molecular targets and metabolic pathways used by microorganisms and cancer cells.

## *Suggested Readings*

Canellos GP, Anderson JR, Propert KJ, et al. Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. N Engl J Med 1992;327:1478–1484. (*These antineoplastic drug combinations remain the standard of care for advanced Hodgkin's disease.*)

Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27–55. (*Detailed analysis of models for synergistic, antagonistic, and additive drug combinations.*)

Espinal MA, Laszlo A, Simonsen L, et al. Global trends in resistance to antituberculosis drugs. N Engl J Med 2001;344:1294–1303. (*Highlights the importance of combination therapy in the treatment of tuberculosis.*)

Harvey RJ. Synergism in the folate pathway. Rev Infect Dis 1982;4:255–260. (*Describes the kinetics of synergism between trimethoprim and the sulfonamides.*)

Koynov KD, Tzekova VI, Velikova MT, et al. Cisplatin, vinblastine and bleomycin in the treatment of disseminated testicular cancer. Int Urol Nephrol 1993;25: 389–394. (*This antineoplastic drug combination remains the standard of care for metastatic testicular cancer.*)

Murphy EL, Collier AC, Kalish LA, et al. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann Intern Med 2001;135:17–26. (*Highlights the importance of combination therapy in the treatment of HIV.*)

# VI

# Principles of Inflammation and Immune Pharmacology

# 39

# Principles of Inflammation and the Immune System

Ehrin J. Armstrong, Harris S. Rose, and Lloyd B. Klickstein

**Introduction**
**Case**
**Overview of the Immune System**
  Innate Immunity
  Adaptive Immunity
    *Major Histocompatibility Complex*
    *Immune Diversity*
    *Tolerance*
    *Humoral and Cellular Immunity*
**Chemical Mediators of Inflammation**
  Histamine
  Complement
  Eicosanoids
  Cytokines
  Other Agents
**The Inflammatory Response**
  Dilation of Vessels
  Recruitment of Cells
  Chemotaxis
  Phagocytosis
  Resolution
**Chronic Inflammation**
**Suggested Readings**

## INTRODUCTION

Inflammation and the immune system are closely intertwined. Inflammation consists of a complex web of responses to tissue injury and infection. It is characterized by the classic signs of *rubor* (redness), *calor* (heat), *tumor* (swelling), *dolor* (pain), and *functio laesa* (loss of function). The immune system consists of the cells and soluble factors, such as antibody and complement, that mediate the inflammatory response; these cells and factors both eliminate the inciting inflammatory stimulus and initiate the process of immunologic memory.

A normal inflammatory response is an acute process that resolves after removal of the inciting stimulus. Diseases of inflammation and immunity can occur when the normal inflammatory response progresses to chronic inflammation, either because of a long-term inappropriate response to a stimulus (for example, allergies) or because the offending agent is not removed (for example, chronic infection, transplantation, and autoimmunity).

Two pharmacologic strategies are used to target the pathophysiology of immune diseases. The first strategy involves modification of the signaling mediators of the inflammatory process or suppression of components of the immune system. This is the rationale for drugs that affect eicosanoid pathways (Chapter 40, Pharmacology of Eicosanoids), histamine (Chapter 41, Histamine Pharmacology), and cells of the immune system (Chapter 42, Pharmacology of Hematopoietic and Immune System Stimulation and Chapter 43, Pharmacology of Immunosuppression). This approach (because it is dependent on molecular understanding of the relevant pathways) is still in its infancy, but promises to yield a number of new drugs in the foreseeable future.

The second pharmacologic approach, used in diseases such as peptic ulcer disease (Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease), asthma (Chapter 45, Integrative Inflammation Pharmacology: Asthma), and gout (Chapter 46, Integrative Inflammation Pharmacology: Gout), involves modification of the underlying pathophysiologic stimulus, thus removing the impetus for inflammation. The difference between these two approaches is, at times, indistinct, and will continue to blur as the pathophysiology of chronic disease is better understood at a molecular level.

This chapter provides sufficient background in the physiology of inflammation and the immune system to understand the subsequent chapters in this section of the book. The treatment of this subject is necessarily brief, with an emphasis on pharmacologically relevant targets of the inflammatory response. The chapter is organized into four parts. First, a general overview of the immune system is presented. Second, the molecular signals mediating cellular communication and inflammation are introduced. Third, the immune and inflammatory cells and signaling molecules are discussed in the context of an integrated inflammatory response. Fourth, chronic inflammation, a pathologic state that is often associated with autoimmunity, is presented. For a more exhaustive presentation of this rapidly changing subject, refer to the Suggested Readings at the end of this chapter and to other chapters in this section.

## Case

Mark is stressed—he has to take the USMLE in 2 weeks, and he has barely begun to study. Throwing aside any pretense of a balanced lifestyle, Mark begins to study constantly from the moment he wakes up, even during meals and in the bathroom. During this mad rush, Mark is, at one point, studying his gastroenterology class notes while sitting on the toilet. Flipping madly through the looseleaf pages, Mark accidentally cuts his thumb on one of the page corners. Fearing the worst, but thinking he lacks the time to clean his thumb properly, Mark continues studying in his room that day. Over the next 5 hours, Mark's thumb becomes progressively swollen, warm, red, and tender. Mark, remarkably, retains focus and continues to study through the evening. By that night, however, Mark develops a fever and increased swelling in the thumb. By the third day, pus builds up at the site. By the fourth day, however, Mark's body seems to have gotten the better of the offending agent. The swelling decreases, the site loses its distinctive angry red appearance, and Mark's fever abruptly subsides. Relieved that he has not become a casualty of his own procrastination, Mark continues studying and performs well on his exam, not the least because his wound has provided him with fundamental insights into immunology.

### QUESTIONS

1. What initial changes in the vasculature accounted for the immediate swelling of Mark's thumb?
2. What chemical signals mediated the inflammatory response in Mark's thumb?
3. What mediators accounted for Mark's fever?
4. Which pathways in the inflammatory cascade can be interrupted using currently available pharmacologic agents?

## OVERVIEW OF THE IMMUNE SYSTEM

The fundamental role of the immune system is to distinguish self from nonself. "Nonself" can consist of an infectious organism, a transplanted organ, or an endogenous tissue that is mistaken for something foreign. Because protection against infection is the classic role of the immune system, the phrases "infection" and "infectious agent" are generally used to denote the inciting stimuli for an immune response. It should be understood, however, that the immune system can be stimulated to react against any nonself entity.

Skin and barrier agents form the first line of defense against any infection. (In the introductory case, Mark's infection occurred only after he cut his skin.) Once an offending agent penetrates these barriers, the immune system mounts a response. The immune response consists of innate and adaptive responses. **Innate** responses are stereotyped reactions to a stimulus (e.g., release of histamine, phagocytosis of a bacterium). In some cases, innate responses are sufficient to neutralize the offending agent. Cells of the innate immune system, especially antigen-presenting cells, can also process the offending agent into small fragments; this processing is necessary for activation of the adaptive immune system. **Adaptive** responses are neutralizing reactions that are specific to the offending agent (e.g., antibodies, cytotoxic T cells). In general, then, *the innate immune system initiates and activates the response to an offending agent, while the adaptive immune system creates a response that specifically neutralizes or kills that agent.*

There are many different cell types in the immune system, and these cell types interact in a complex web of signaling and communication to create the overall response. The cells of the immune system derive from two types of stem cells in the bone marrow: **myeloid** stem cells and **lymphoid** stem cells. (The lymphoid stem cell is sometimes called the **common lymphoid stem cell** because it gives rise to both B cells and T cells.) Myeloid stem cells give rise to precursors of cells of the innate immune system, whereas lymphoid stem cells generate precursors of cells of the adaptive immune system. Figure 39-1 shows the myeloid and lymphoid stem cells and the mature cell types into which their respective precursor cells differentiate. The derivation of these cell types is also discussed in Chapter 42.

## INNATE IMMUNITY

Cells of the innate immune system are the first responders to an offending agent that has penetrated the skin or another barrier (Table 39-1). Innate immune cells perform three important tasks. First, these cells defend against bacterial and parasitic infections, either by neutralizing the infectious agent with secreted cytotoxic proteins, or by phagocytosis (engulfing) of the bacterium or parasite. Second, phagocytosis of the offending agent initiates proteolytic digestion of microbial macromolecules to fragments (antigens) that are then displayed, together with major histocompatibility complex (MHC) class II proteins, on the surface of antigen-presenting cells. In turn, these antigen-presenting cells, which include macrophages and dendritic cells, activate cells of the adaptive immune system. Third, the offending agent stimulates innate immune cells to secrete numerous cytokines (see below) that further amplify the immune response. The major cell types of the innate immune system include **granulocytes** (neutrophils, eosinophils, and basophils), mast cells, and **antigen-presenting cells** (macrophages and dendritic cells).

"Granulocyte" is a descriptive term based on the appearance of the cytoplasmic granules within these cells. **Neutrophils,** the most abundant cell type of the innate immune system, are phagocytic cells that are primarily responsible for defense against bacterial infection. These cells envelop invading bacteria in phagocytic vesicles and destroy the bacteria within these vesicles using such endogenous enzymes

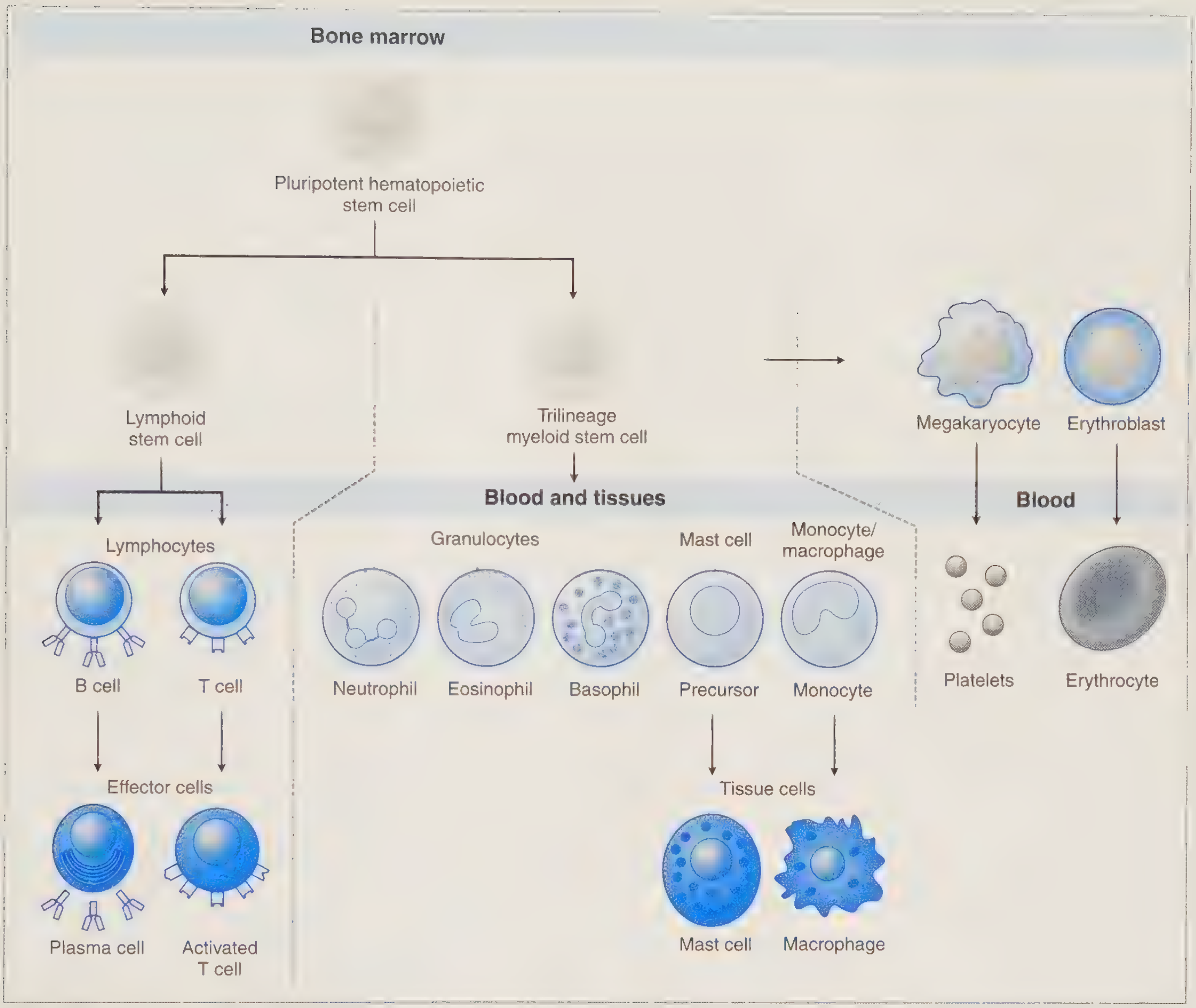

Bone marrow
Pluripotent hematopoietic stem cell
Lymphoid stem cell
Trilineage myeloid stem cell
Megakaryocyte
Erythroblast
Blood and tissues
Blood
Lymphocytes
Granulocytes
Mast cell
Monocyte/ macrophage
B cell
T cell
Neutrophil
Eosinophil
Basophil
Precursor
Monocyte
Platelets
Erythrocyte
Effector cells
Tissue cells
Plasma cell
Activated T cell
Mast cell
Macrophage

**Figure 39-1. Development of Cells of the Immune System.** All hematopoietic cells develop from the pluripotent hematopoietic stem cell. This cell gives rise to the lymphoid stem cell and the trilineage myeloid stem cell. The lymphoid stem cell and its progenitor cells (not shown) give rise to mature lymphocytes (B cells and T cells), the cells that mediate adaptive immune responses. When exposed to specific antigens, B cells differentiate into antibody-producing plasma cells, and T cells assume an activated phenotype. The myeloid stem cell and its progenitor cells, including megakaryocytes, erythroblasts, and myeloid precursors (not shown), proliferate and differentiate into mature neutrophils, eosinophils, basophils, mast cells, monocytes, platelets, and erythrocytes. In the tissues, monocytes differentiate into macrophages and mast cell precursors differentiate into mast cells. (See Fig. 42-1 for more details about the differentiation of cell lineages in the bone marrow.)

as myeloperoxidase. **Eosinophils** are circulating granulocytes that are primarily involved in defense against parasitic infections. Because parasites are often too large to engulf, eosinophils attach to a parasite's exterior and secrete cytotoxic substances directly on the parasite. Both **basophils** (circulating) and mast cells (tissue-resident) bind IgE antibody, display this IgE on the cell surface, and maintain histamine-containing granules that are released when exogenous antigen binds to and crosslinks the IgE. Basophils and mast cells are important in allergic responses. Eosinophils and basophils are so named because they have eosinophilic and basophilic patterns, respectively, when stained with Wright–Giemsa stain.

Antigen-presenting cells (APCs) process the macromolecules (especially proteins) of an invading agent to display the processed fragments on the surface of the APC. In this form, the fragments serve as molecular fingerprints used by cells of the adaptive immune system to recognize the invading agent. APCs are important initiators of immune responses because, in addition to displaying nonself antigens to T cells (see below), they provide the costimulatory signals that are necessary for T cell activation. This concept of **costimulation,** in which two separate signals are required to initiate an immune response to a stimulus, is discussed further in Chapter 43.

Monocytes that exit the bloodstream and take up residence in the tissues can differentiate into **macrophages.** As "professional APCs," macrophages process and present antigenic fragments of an invading pathogen for recognition by T cells. The ability of macrophages to envelop and destroy pathogens is enhanced by other components of the immune system, including antibody-mediated opsonization and cytokine-

**TABLE 39-1** Cells of the Immune System

| CELL TYPE | FUNCTION |
|---|---|
| **Innate Immunity** | |
| Macrophage | Tissue-resident cell, derived from monocyte<br>Phagocytoses cellular and foreign debris<br>Involved in chronic inflammation<br>Antigen-presenting cell |
| Dendritic cell | Transports and presents antigen to T cells in lymph nodes<br>Antigen-presenting cell |
| Neutrophil | Phagocytoses and kills invading pathogens, especially bacteria |
| Eosinophil | Defends against parasites |
| Basophil<br>Mast cell | Release histamine, leukotrienes and other mediators after exposure to antigen |
| **Adaptive Immunity** | |
| Cytotoxic T cell ($T_C$) | Effector of cellular adaptive immunity |
| Helper T cell ($T_H$) | Controls adaptive immune responses |
| B cell | Synthesizes and secretes antibody<br>Antigen-presenting cell |

enhanced killing ability. In addition, macrophages produce cytokines such as TNF-α that modify immune responses. **Dendritic cells** are antigen-presenting cells that, in their mature form, are found primarily in the T cell areas of lymphoid tissue. Dendritic cells are the most important APCs for the initiation of adaptive immune responses. Immature dendritic cells reside in nonlymphoid tissues, where they stand ready to engulf and process foreign antigens; the dendritic cells then transport these antigens to lymphoid tissues and present the antigens to T cells.

## ADAPTIVE IMMUNITY

Innate immune cells respond to common determinants that are present on many invading agents (for example, lipopolysaccharide (LPS) present in the outer membrane of Gram-negative bacteria). In this role, innate immune cells use **pattern recognition** to phagocytose a class of infectious agents rather than a particular infectious agent. In contrast, adaptive immune cells mount a specific response to the three-dimensional conformation of a particular antigen, referred to as an **epitope.** From a teleological perspective, innate immunity provides a broad gating function, attempting to counteract harmful effects of foreign invaders in a rapid manner and to determine whether an infectious agent should be further attacked by adaptive immunity, while adaptive immunity provides a specialized response that is specific to the invading infectious agent. Innate immune cells lack memory; they respond in the same way, and to the same extent, to repeated infections with the same agent. In contrast, adaptive immune cells mount a faster and more intense response upon re-exposure to an infectious agent.

The main features of the adaptive immune system, specificity to foreign antigens and tolerance to self-antigens, rely on two factors. First, there must be a mechanism to generate a specific response that will attack a foreign antigen. Second, adaptive immune cells must be able to distinguish native (self) cells and soluble factors from foreign (nonself) cells and soluble factors. The first property is provided by **major histocompatibility complex** (MHC) proteins along with somatic gene recombination in T cells and B cells, whereas the second property is provided by regulated immune cell development and costimulation.

### Major Histocompatibility Complex

MHC proteins are transmembrane proteins that bind and display on their surface proteolytically degraded protein fragments and, in some cases, glycolipid antigens. There are two classes of MHC proteins, MHC class I and MHC class II. MHC class I proteins primarily display fragments of cytosolic proteins (Fig. 39-2). All nucleated cells express MHC class I proteins; the repertoire of protein fragments displayed by MHC class I proteins on a cell provides a fingerprint for all the proteins expressed within that cell. If a cell is expressing a recognizable pattern of proteins, then it will not be attacked by the immune system. However, if foreign (for example, viral) proteins are being generated within the cytosol of the cell, then proteolytic fragments of those viral proteins will be displayed on MHC class I proteins at the surface of the cell, and the immune system will recognize that cell as virally infected. Antigens presented by MHC class I proteins are recognized by T cells bearing the cell surface marker CD8.

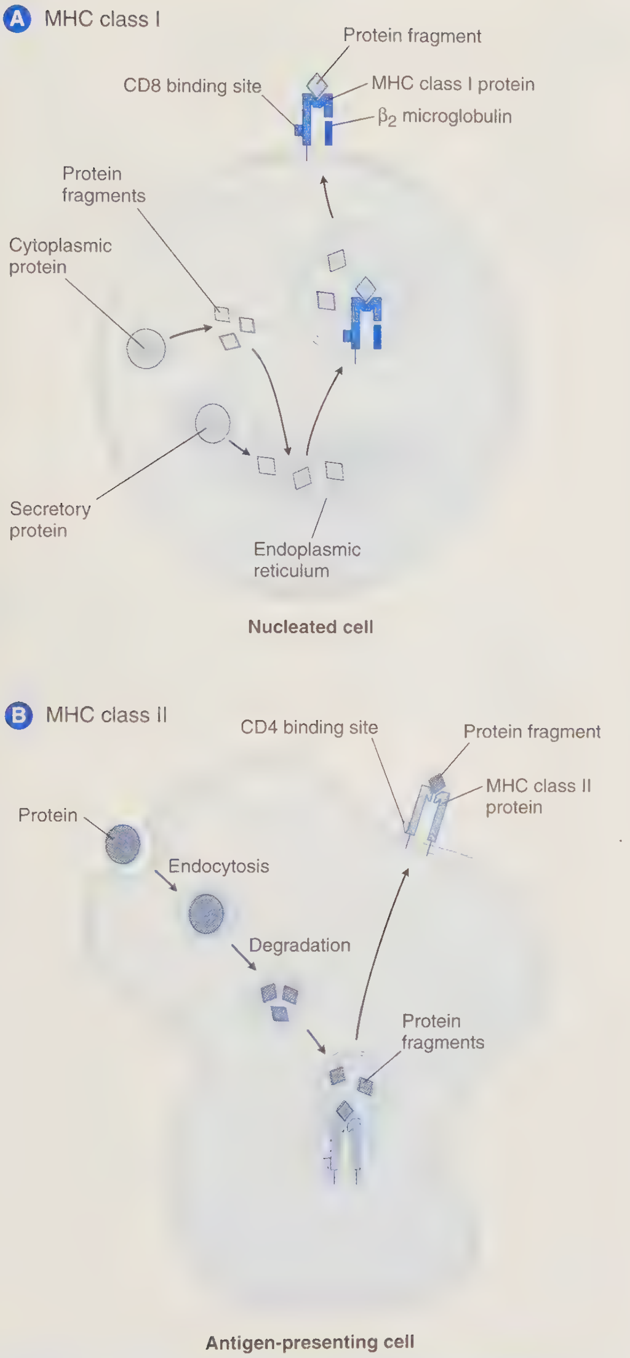


**Figure 39-2. Class I and Class II Major Histocompatibility Complex Proteins.** **A.** Cytoplasmic proteins are proteolytically degraded in the cytosol, and the protein fragments are transported to the endoplasmic reticulum (ER). Proteins destined for secretion are degraded directly in the ER. MHC class I protein, in association with $\beta_2$ microglobulin, binds a fragment of the degraded cytoplasmic or secretory protein in the ER. The MHC class I:protein fragment complex is transported to the cell surface, where it serves as a fingerprint for the diversity of proteins expressed by that cell. The CD8 binding site on MHC class I ensures that the class I protein:antigen complex interacts only with cytotoxic T cells, which express CD8. All nucleated human cells express MHC class I proteins. **B.** Antigen-presenting cells phagocytose and degrade bacteria and other foreign agents, generating protein fragments that bind to MHC class II protein in the ER. The MHC class II:protein fragment complex is transported to the cell surface, where it serves to display all the potentially nonself antigens that have been ingested by that cell. The CD4 binding site on MHC class II ensures that the class II protein:antigen complex interacts only with helper T cells, which express CD4. Professional antigen-presenting cells (B cells, macrophages, and dendritic cells) are usually the only cell types that express MHC class II proteins, but other cells can be induced to express class II proteins and present antigens under some circumstances.

MHC class II proteins display protein fragments derived from endocytic vesicles. In contrast to class I proteins, which are expressed on all nucleated cells, MHC class II proteins are expressed mostly on antigen-presenting cells (e.g., macrophages and dendritic cells), although some other cell types can be induced to express MHC class II proteins. Endocytic vesicles contain antigenic protein fragments derived from infectious agents after phagocytosis and proteolytic processing of those agents. Therefore, the protein fragments expressed on MHC class II proteins identify extracellular foreign agents (e.g., bacteria). As discussed below, T cells expressing the cell surface marker CD4 recognize antigens presented by MHC class II proteins. In the process, these T cells activate the antigen-presenting cells to produce soluble factors called cytokines and chemokines that, in turn, aid the T cells in responding to the antigen. In general, then, *the protein fragments bound to MHC class I identify infected cells, whereas the fragments bound to MHC class II identify infectious agents.* However, because of the phenomenon of cross presentation, some proteins generated in the cytosol can be presented by MHC class II to $CD4^+$ T cells, and some phagocytosed antigens can be presented by MHC class I to $CD8^+$ T cells.

## Immune Diversity

MHC proteins provide a means for distinguishing infected cells and infectious agents from uninfected cells; somatic gene recombination provides a means for generating a specific response to an infection. Cells of the adaptive immune system undergo **gene rearrangements** and RNA splicing to generate antibodies (B cells) and specific cell surface receptors (T cells) (both of these cell types are discussed below). By recombination, **immunoglobulin** and **T cell receptor** genes semirandomly create millions of modular three-dimensional protein structures (referred to as variable regions) that, in the aggregate, can recognize almost any structure. This is the mechanism by which the immune system can generate an astounding diversity of immune responses.

## Tolerance

The diversity in the variable regions of immunoglobulins and T cell receptors creates the potential that some of these molecules could recognize and attack native proteins. In a process referred to as **tolerance,** cells of the immune system undergo a carefully regulated series of steps during development to ensure that mature immune cells do not recognize native proteins. In addition, **costimulation**—the requirement for multiple simultaneous signals to initiate an immune response—ensures that stimulation of a single immune receptor does not activate a damaging immune reaction. As discussed in more detail in Chapter 43, defects in tolerance can lead to autoimmune diseases.

## Humoral and Cellular Immunity

Adaptive immunity is generally divided into **humoral immunity** and **cellular immunity.** The primary cells mediating these branches of the immune system are referred to as **B cells** and **T cells,** respectively (Table 39-1). The humoral response involves the production of **antibodies** specific for an antigen. These antibodies are secreted by plasma cells (differentiated B cells) and are, therefore, most effective against such extracellular infectious agents as bacteria. The cellular response involves activation and clonal expansion of T cells that recognize a specific antigen. Some T cells recognize infected cells and then lyse those cells using cytotoxic proteins called **perforins** and **granzymes.** Cellular immune responses are, therefore, effective against many intracellular infectious agents, such as viruses.

In addition to their role in cellular immunity, T cells control the extent of immune responses. Each T cell evolves so that it is activated by only one specific MHC:antigen complex. All T cells express an MHC:antigen-specific T cell receptor (TCR). T cells are divided into cytotoxic T cells ($T_C$) and helper T cells ($T_H$), based on the type of coreceptor expressed and the function imparted by that coreceptor (Fig. 39-3).

**$T_C$ cells** are the *mediators* of cellular adaptive immunity. These cells express the CD8 coreceptor, which recognizes a constant (i.e., antigen-independent) domain on MHC class I proteins. This coreceptor function allows the antigen-specific TCR on $T_C$ cells to bind a specific class I MHC:antigen complex with sufficiently high affinity that the $T_C$ cell is activated by the cell expressing the class I MHC:antigen complex. Specific activation of the $T_C$ cell initiates a chain of events, including the secretion of membrane-penetrating perforins and apoptosis-inducing granzymes, that results in the death of the cell displaying the foreign antigen.

**$T_H$ cells** are primarily the *regulators* of adaptive immunity. $T_H$ cells are identified by their expression of the CD4 coreceptor, which recognizes an antigen-independent domain on MHC class II proteins. This coreceptor function allows the antigen-specific receptor on $T_H$ cells to bind a specific class II MHC:antigen complex with sufficiently high affinity that the $T_H$ cell is activated by the antigen-presenting cell. In addition to initiating and strengthening the immune response, $T_H$ cells control the type of immune response by producing one or another set of cytokines. The $T_H$ cells can be divided into $T_H1$ and $T_H2$ subtypes based on the cytokines produced by the cells. $T_H1$ cells characteristically produce IFN-γ and IL-2, and these cytokines influence the development of cell-mediated immune responses of both $CD8^+$ $T_C$ cells and other $CD4^+$ $T_H$ cells. In contrast, $T_H2$ cells characteristically produce IL-4, IL-5, and IL-10, and these cytokines enhance antibody production by B cells. The $T_H2$ cell subtype is more often associated with autoimmunity (see Chapter 43). In addition to regulating adaptive immunity, $T_H$ cells can mediate immunity by secreting cytokines that activate phagocytic cells to kill infecting microbes more efficiently.

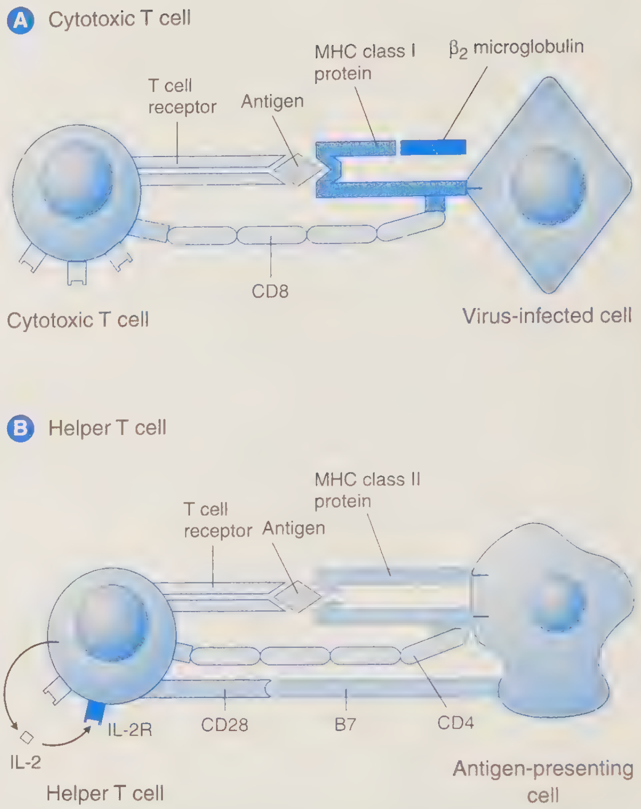


**Figure 39-3. Activation of Cytotoxic and Helper T Cells.** T cells mediate and regulate the cellular immune response. **A.** Cytotoxic T cells ($T_C$) are the primary *mediators* of cellular immunity. These cells express T cell receptors (TCR) and CD8. The TCR identifies nonself antigens bound to MHC proteins, whereas the CD8 ensures that $T_C$ cells interact only with cells expressing MHC class I proteins. In the example shown, the interaction of a $T_C$ cell with the MHC class I protein of a virus-infected cell leads to activation of the $T_C$ cell and subsequent killing of the virus-infected cell. **B.** Helper T cells ($T_H$) are the primary *regulators* of cellular immunity. These cells express TCR and CD4. CD4 binds to MHC class II proteins on antigen-presenting cells (APC); this interaction ensures that $T_H$ cells interact only with cells expressing MHC class II proteins. An additional degree of specificity is provided by the interaction of CD28 on $T_H$ cells with proteins of the B7 family on APC; this "costimulatory signal" is required for $T_H$ activation. In the example shown, the interaction of a $T_H$ cell with the MHC class II and B7 proteins of an antigen-presenting cell leads to activation of the $T_H$ cell. The activated $T_H$ cell secretes IL-2 and expresses the IL-2 receptor (IL-2R); this autocrine pathway stimulates further $T_H$ cell proliferation and activation. IL-2 (and other cytokines secreted by the $T_H$ cell) activate not only $T_H$ cells, but also $T_C$ cells and B cells.

## CHEMICAL MEDIATORS OF INFLAMMATION

The discussion to this point has focused on the cells of the immune system and their roles in mounting an immune response. Equally important, and the main targets of pharmacologic intervention, are the molecular mediators of immune cell activity. The discussion below highlights endogenous molecules that regulate the inflammatory process. (Signaling pathways for immune cells are discussed mainly in Chapter 43, although there is some overlap among the endogenous mediators of inflammation and immunity, especially among the cytokines.) The list of mediators is long (Table 39-2), and essentially all of these signaling systems have been explored as potential pharmacologic targets. Only those most crucial to inflammation and those for which therapies already exist are discussed in detail.

**TABLE 39-2** Chemical Mediators of the Inflammatory Response

| RESPONSE | MEDIATORS |
|---|---|
| Vasodilation | Prostaglandins (PG) $PGI_2$, $PGE_1$, $PGE_2$, $PGD_2$<br>Nitric oxide (NO) |
| Increased vascular permeability | Histamine<br>C3a, C5a (complement components)<br>Bradykinin<br>Leukotrienes (LT), especially $LTC_4$, $LTD_4$, $LTE_4$<br>Platelet-activating factor<br>Substance P<br>Calcitonin gene-related peptide (CGRP) |
| Chemotaxis and leukocyte activation | C5a<br>$LTB_4$, lipoxins (LX) $LXA_4$, $LXB_4$<br>Bacterial products |
| Tissue damage | Neutrophil and macrophage lysosomal products<br>Oxygen radicals<br>NO |
| Fever | IL-1, IL-6, TNF<br>$LTB_4$, $LXA_4$, $LXB_4$ |
| Pain | $PGE_2$, $PGI_2$<br>Bradykinin<br>CGRP |

## HISTAMINE

**Histamine** is one of the initiators of the inflammatory response. It is constitutively synthesized and stored in the granules of mast cells and basophils. These cells migrate through tissue on a continual basis. Any injury, from physical trauma to microbial invasion, stimulates mast cells to release histamine granules into the interstitium. Histamine is referred to as a "vasoactive amine" because its inflammatory effects occur mainly on the vasculature—histamine release stimulates dilation of arterioles and postcapillary venules, constriction of veins, and contraction of endothelial cells. These effects are responsible for the early changes in hemodynamics and vascular permeability, discussed below. A number of pharmacologic agents modify histamine signaling; these agents are discussed in Chapter 41, Histamine Pharmacology and Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease.

## COMPLEMENT

**Complement** comprises a system of serine proteases that is one of the first innate mechanisms to be activated in response to injury. The complement system can be activated by antigen–antibody interactions (the classical pathway), by direct interactions with foreign surfaces (the alternative pathway), or by interactions with certain complex carbohydrates (the lectin pathway). In each pathway, a series of proteolytic reactions converts a complement precursor protein, referred to by the letter "C" followed by a number (for example, C3), into its active form, indicated by the letter "a" or "b" (for example, C3a). The general scheme of this pathway is analogous to that of the coagulation cascade (see Chapter 21, Pharmacology of Hemostasis and Thrombosis): precursor proteins are proteolytically cleaved to active products, which then regulate the actions of the cascade.

After activation, complement triggers further inflammatory responses by two mechanisms. First, several cleavage products of the complement cascade are potent stimulators of inflammation. For example, C3b is an important opsonin, whereas C3a and C5a mediate leukocyte chemotaxis. Second, the final step in complement activation is the assembly of the **membrane attack complex.** This complex of complement proteins forms large pores in the outer membrane of Gram-negative bacteria, leading to lysis of the bacteria. A large number of complement regulatory proteins, both soluble and on the cell surface, carefully govern and localize complement activation to the site of inflammation. Inhibitors of complement activation are under development as potential inhibitors of the tissue injury associated with inappropriate inflammatory responses.

## EICOSANOIDS

**Eicosanoids** are metabolites of arachidonic acid, a fatty acid component of phospholipids in the inner leaflet of the plasma membrane of many cell types. Such inflammatory mediators as cytokines and complement are able to stimulate the enzymatic release of arachidonic acid from the plasma membrane. A number of biochemical reactions ensue, resulting in the formation of prostaglandins, leukotrienes, and other eicosanoids. Notably, certain arachidonic acid derivatives are proinflammatory, whereas others serve to limit the inflammatory process. This underscores the fact that acute inflammation is a self-limited process and that the process of pathogen destruction is intimately tied to the process of tissue repair. Chapter 40, Pharmacology of Eicosanoids, is devoted to an in-depth discussion of eicosanoid physiology, pathophysiology, and pharmacology.

### CYTOKINES

**Cytokines** are proteins that act in a paracrine manner to regulate leukocyte activity. **Interleukins** are cytokines secreted by cells of the hematopoietic lineage. Interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) are two cytokines elaborated as part of an acute inflammatory response; these cytokines were two of the mediators responsible for Mark's fever. Other notable cytokines include the hematopoietic growth factors granulocyte–monocyte colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) (see Chapter 42).

Because cytokines affect the proliferation and function of cells that mediate innate and adaptive immune responses, selective inhibition or stimulation of cytokine actions has the potential to modulate immune and inflammatory responses. Pharmacologic uses for cytokine and anticytokine therapies are discussed in Chapter 42 and Chapter 43, respectively.

### OTHER AGENTS

As noted in Table 39-2, a number of other signaling systems are also used to coordinate the inflammatory response. These include kinins, platelet-activating factor, nitric oxide, oxygen radicals, and other leukocyte and bacterial products released during phagocytosis. Although pharmacologic agents are being developed to modulate each of these pathways, there are, as yet, no approved anti-inflammatory drugs that specifically interrupt these pathways.

## THE INFLAMMATORY RESPONSE

The cells and soluble mediators of the immune system interact with one another to generate the **inflammatory response,** which typically occurs in four phases. First, the vasculature around a site of injury reacts to recruit cells of the immune system. Second, circulating immune cells migrate from these vessels into the injured tissues, and the mechanisms of innate and adaptive immunity (see above) serve to neutralize and remove the inciting stimulus. Next, the process of repair and tissue healing ensues, and the acute inflammatory process is terminated. If the events of inflammation are not halted but continue to smoulder, chronic inflammation can occur.

### DILATION OF VESSELS

Within hours of being cut, Mark's thumb begins to exhibit the five classic signs of inflammation presented in the Introduction. Initially, these signs and symptoms result from alterations in vascular hemodynamics at the site of injury. Injury to a tissue causes the release of inflammatory mediators (discussed above) that dilate arterioles and postcapillary venules; in turn, vasodilation leads to increased blood flow to the site of injury, causing the clinical signs of redness and warmth. Inflammatory mediators also cause contraction of vascular endothelial cells, leading to increased capillary permeability and to the development of an exudate (i.e., interstitial fluid with a high protein content); in turn, the exudate causes the clinical manifestations of swelling and pain from increased tissue pressure.

### RECRUITMENT OF CELLS

Increased vascular permeability also allows cells from the blood to enter the interstitium. Cellular migration out of the blood is not random; rather, leukocyte recruitment is orchestrated to optimize clearing of the infection or repair of the injured tissue (Fig. 39-4). At the onset of an inflammatory response, the endothelial cells at the site of injury are activated to express adhesion molecules that bind specific receptors expressed by leukocytes. For example, intercellular adhesion molecules (**ICAMs**) expressed by activated endothelial cells bind integrins expressed on the cell surface of leukocytes. This interaction causes the leukocytes, which normally roll along the surface of the endothelium by means of loose, transient binding interactions, to adhere tightly to the activated endothelium at the site of injury. The adherent leukocytes then bind other endothelial cell receptors that promote **transmigration** (diapedesis) of the leukocytes from the vasculature into the interstitium. Specificity of the immune response is achieved according to the pattern of adhesion molecules expressed by the activated endothelium and the various types of leukocytes; for example, neutrophils dominate the early inflammatory response, while monocytes predominate after 24 hours.

### CHEMOTAXIS

Once the cells of the immune system have crossed the endothelial barrier, they can migrate through the interstitium to the specific site of injury or infection. Immune cell targeting is accomplished by the process of **chemotaxis,** or chemical signaling. Inflammatory mediators released at the site of injury, such as C3a and leukotriene B4 ($LTB_4$), create a chemical gradient to which the leukocytes respond, allowing them to move preferentially toward the site of the inflammatory reaction.

### PHAGOCYTOSIS

Upon their arrival at the site of injury or infection, the neutrophils, macrophages, and other cells of the immune system are ready to perform their duties. However, these cells require one further stimulus to activate their killing machinery. Foreign substances must be coated by an opsonin before they can be ingested (phagocytosed) by leukocytes. **Opsonins** are molecular adaptors that coat foreign surfaces and signal leukocytes that a particle should be attacked. The major opsonins consist of complement, immunoglobulins (antibodies), and **collectins** (plasma proteins that bind to certain microbial carbohydrates). The interaction of a

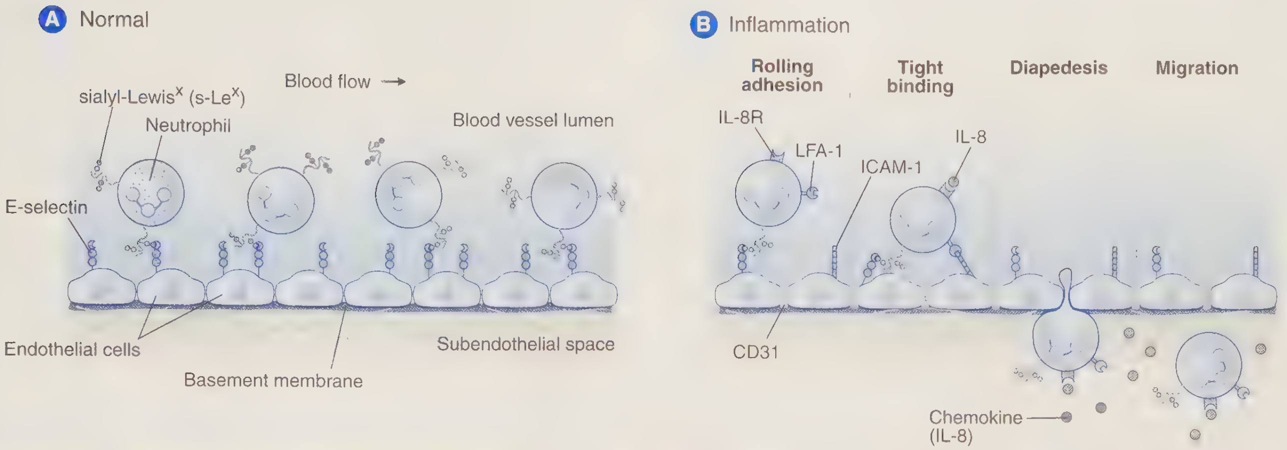


**Figure 39-4. Overview of the Inflammatory Response.** **A.** Leukocytes circulating in the blood interact with selectins expressed on the surface of vascular endothelial cells. In the absence of inflammation, the interaction between leukocytes and endothelial cells is weak, and leukocytes either flow past or roll along the endothelium. Neutrophil rolling is mediated by the interaction between endothelial cell E-selectin and neutrophil sialyl-Lewis$^x$ (s-Le$^x$). **B.** During the inflammatory response, endothelial cells upregulate their expression of intercellular adhesion molecules (ICAMs). ICAM expression increases the potential for strong binding interactions between leukocytes and the activated endothelial cells. For example, ICAM-1 on endothelial cells binds tightly to LFA-1 on neutrophils. The enhanced cell–cell interaction leads to margination of leukocytes onto endothelial cell surfaces and initiates the process of leukocyte diapedesis and transmigration from the vascular space into extravascular tissues. Leukocytes migrate through injured tissue in response to such chemokines as IL-8, which are inflammatory mediators released by injured cells and by other immune cells that have already reached the site of injury.

phagocytic cell with an opsonized particle initiates the engulfment and destruction of the offending agent. This step is also the crucial point of interaction between innate and adaptive immunity. Antigen-presenting cells process engulfed particles and present their antigens to B cells and T cells, which then react to the antigens. In the introductory case, Mark's cut presumably allowed Gram-negative enteric bacteria to penetrate across his skin barrier, leading to infection. The presence of these bacteria initiated an inflammatory response that included phagocytosis of bacteria by APCs, presentation of bacterial antigens to $T_H$ cells, activation and expansion of $T_H$ cells, $T_H$ cell activation of further APC-mediated phagocytosis, and synthesis and secretion of antibodies specific for the bacteria.

## RESOLUTION

Tissue repair and the re-establishment of homeostasis are the final events in the acute inflammatory response. The same mediators that activate inflammation also initiate a cascade of tissue repair; this process is mediated by the release of growth factors and cytokines including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor-2 (bFGF-2), transforming growth factor β1 (TGF-β1), IL-1 and TNF-α. These factors act as mitogens for endothelial cells and fibroblasts, and ultimately stimulate healing and scar formation through angiogenesis (formation of new blood vessels) and the formation of granulation tissue. In the introductory case, the granulation tissue and eventual scar will be the only record of Mark's acute inflammatory event. Of note, angiogenesis can be a pathologic state when it is associated with tumor growth, and pharmacologic inhibitors of angiogenesis are under development as potential antineoplastic agents.

## CHRONIC INFLAMMATION

*Chronic inflammation is a pathologic state characterized by the continued and inappropriate response of the immune system to an inflammatory stimulus.* Chronic inflammation accounts for the symptoms of many autoimmune diseases and may be an important cause of organ transplant rejection. In contrast to the acute inflammatory response, which is dominated by neutrophils, one of the hallmarks of chronic inflammation is the predominance of macrophages. Activated macrophages secrete such inflammatory mediators as proteases and eicosanoids, as well as collagenases and growth factors. These secreted products initiate and maintain a cycle of tissue injury and repair, leading to tissue remodeling. Over time, chronic inflammation can cause relentless tissue destruction. Promising areas of treatment for chronic inflammation include the use of cytokine inhibitors to neutralize mediators of the signaling cascades that perpetuate chronic inflammation. These agents are discussed in Chapter 43.

### ■ *Suggested Readings*

Delves PJ, Roitt IM. Advances in immunology: the immune system—first of two parts. N Engl J Med 2000;343:37–49. (*This article and subsequent articles in the series provide an excellent general introduction to immunology.*)

Janeway CA, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th ed. New York: Garland Publishing, 2001. (*A general immunology textbook.*)

Pier GB, Lyczak JB, Wetzler L. Immunology, Infection and Immunity. Washington, DC: ASM Press; 2004. (*A detailed text covering current understanding of immunology mechanisms.*)

www.copewithcytokines.de/cope.cgi (*Website that describes all the known actions of cytokines.*)

# 40

# Pharmacology of Eicosanoids

David M. Dudzinski and Charles N. Serhan

Introduction
Case
Physiology of Arachidonic Acid Metabolism
  Generation of Arachidonic Acid
  Cyclooxygenase Pathway
    *Prostaglandins*
    *Thromboxane and Prostacyclin*
  Lipoxygenase Pathway
    *Leukotrienes*
    *Lipoxins*
  Epoxygenase Pathway
  Isoprostanes
  Metabolic Inactivation of Eicosanoids
  Integrated Inflammation Schema
Pathophysiology
  Asthma
  Inflammatory Bowel Disease
  Rheumatoid Arthritis
  Glomerulonephritis
  Cancer
Pharmacologic Classes and Agents
  Phospholipase Inhibitors
  Cyclooxygenase Inhibitors
    *Traditional Nonselective Inhibitors: NSAIDs*
      *Salicylates*
      *Propionic Acid Derivatives*
      *Acetic Acid Derivatives*
      *Oxicam Derivatives*
      *Fenamate Derivatives*
      *Ketones*
    *Acetaminophen*
    *Selection of the Appropriate NSAID*
    *COX-2 Inhibitors*
      *COX-2 Selective Inhibitors*
      *Glucocorticoids*
      *Cytokine Inhibitors*
  Prostanoid Receptor Mimetics
  Thromboxane Antagonists
  Leukotriene Inhibition
    *Lipoxygenase Inhibition*
    *5-Lipoxygenase Activating Protein (FLAP) Inhibition*
    *Leukotriene Synthesis Inhibitors*
    *Leukotriene Receptor Antagonists*
  Lipoxins and Aspirin-Triggered Lipoxins
Conclusion and Future Directions
Suggested Readings

## INTRODUCTION

**Autacoids** are substances that are rapidly synthesized in response to specific stimuli, act quickly at the immediate locality, and remain active for only a short time before degradation. **Eicosanoids** represent a chemically diverse family of arachidonic acid-derived autacoids. Research on eicosanoids continues to reveal their critical roles in cardiovascular, inflammatory, and reproductive physiology. Numerous pharmacologic interventions in eicosanoid pathways—including the nonsteroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors, and leukotriene inhibitors—are useful in the current clinical management of inflammation, pain, and fever. Given the diverse bioactivities of eicosanoids, future research in eicosanoid physiology and pharmacology may lead to the development of new therapeutics for the treatment of inflammatory conditions, autoimmune diseases, asthma, glomerulonephritis, cancer, sleep disorders, and Alzheimer's disease.

## ■ Case

Mrs. D., a 57-year-old Caucasian female, goes to her physician because of joint pain and chronic fatigue. Her history reveals general joint stiffness and pain, especially in the early morning, and pain in the left metatarsophalangeal joint of 3 weeks duration. Mrs. D. is advised to take ibuprofen as needed, and this medication provides relief of her pain for some time.

Two years later, Mrs. D. notes indigestion and a few isolated instances of vomiting "coffee-grounds"-like material. Her physician recommends an upper gastrointestinal endoscopic examination, which reveals gastric mucosal erosion and hemorrhage. Based on this finding, Mrs. D. is advised to discontinue ibuprofen therapy. However, her physician is also concerned about the recent progression in Mrs. D.'s joint stiffness and pain, and refers her to a rheumatology clinic. Mrs. D. reports to the rheumatologist that her pain has progressed to include both feet, both hands and wrists, both elbows, some cervical vertebrae, and the left hip. Over the past few months, she has noted difficulties with basic household tasks and has avoided physical activity. The metacarpophalangeal and proximal interphalangeal joints of both hands are found to be swollen, tender, and warm. Skin nodules are apparent on the extensor surface of both forearms. Laboratory tests show high erythrocyte sedimentation rate (ESR), low hematocrit, and positive rheumatoid factor (an immune complex formed from IgM and autoimmune-reactive IgG produced in joints). Synovial fluid aspirate is notable for leukocytosis.

Because the presentation is consistent with a diagnosis of rheumatoid arthritis, Mrs. D. is started on a course of celecoxib (a COX-2 selective inhibitor), etanercept (a TNF-α antagonist), and prednisone (a glucocorticoid). Over the next several months, Mrs. D.'s joint pain, swelling, and tenderness decrease noticeably. Joint function in the hands is restored, and Mrs. D. is able to resume some physical activity.

## QUESTIONS

1. Why was ibuprofen sufficient to control Mrs. D.'s early symptoms? Explain the rationale for discontinuing ibuprofen.
2. What are the advantages and limitations of each of the classes of drugs to which celecoxib, etanercept, and prednisone belong?
3. How do glucocorticoids modify eicosanoid pathways?

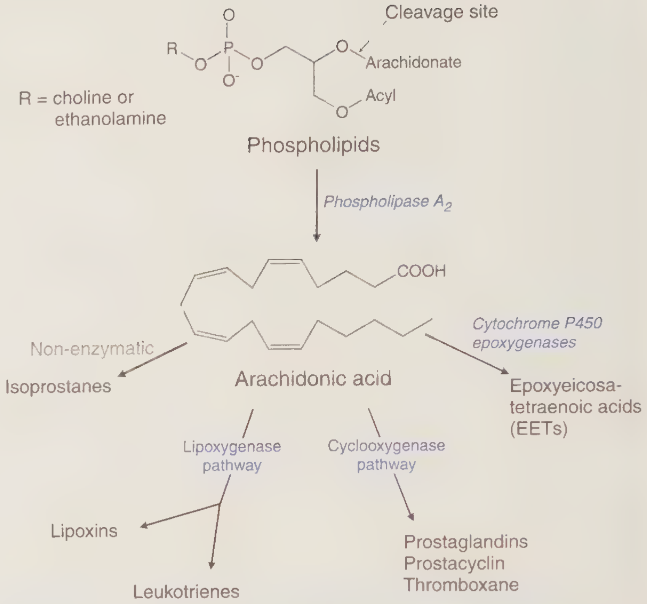


**Figure 40-1. Overview of Arachidonic Acid Metabolism.** Phospholipase $A_2$ acts on the phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) to release arachidonic acid. Free arachidonic acid is then used as the substrate for the cyclooxygenase, lipoxygenase, and epoxygenase pathways. The cyclooxygenase pathway produces prostaglandins, prostacyclin, and thromboxane. The lipoxygenase pathway produces leukotrienes and lipoxins. The epoxygenase pathway produces epoxyeicosatetraenoic acids (EETs). Nonenzymatic peroxidation of arachidonic acid can produce isoprostanes. Phospholipase $A_2$ cleaves the ester bond marked by the arrow to release arachidonic acid.

## PHYSIOLOGY OF ARACHIDONIC ACID METABOLISM

Eicosanoids are crucially involved in a number of metabolic pathways that have diverse roles in inflammation and cellular signaling. All of these pathways center on reactions involving the metabolism of arachidonic acid (Fig. 40-1). The following discussion considers the biochemical steps leading to arachidonic acid synthesis, then discusses the cyclooxygenase, lipoxygenase, epoxygenase, and isoprostane pathways of arachidonic acid metabolism.

## GENERATION OF ARACHIDONIC ACID

**Arachidonic acid** (cis-,cis-,cis-,cis-5,8,11,14-eicosatetraenoic acid), the common precursor to eicosanoids, must be synthesized from the essential fatty acid precursor **linoleic acid** (cis,cis-9,12-octadecadienoic acid), which can be obtained only from dietary sources. In the cell, arachidonic acid does not exist as a free fatty acid, but rather is esterified to the $sn_2$ position of the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine.

Arachidonic acid is released from phospholipids by the enzyme **phospholipase $A_2$** (Fig. 40-1), which hydrolyzes the acyl ester bond. *This important reaction, which represents the first step in the arachidonic acid cascade, is the overall rate-determining step in the generation of eicosanoids.*

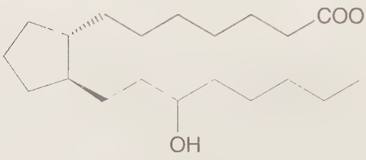
There are membrane-bound and soluble isoforms of phospholipase $A_2$, classified as secretory ($sPLA_2$) and cytoplasmic ($cPLA_2$). The phospholipase $A_2$ isoforms are differentiated based on molecular weight, pH sensitivity, regulation and inhibition characteristics, calcium requirements, and substrate specificity. The existence of multiple isoforms allows variable regulation of the enzyme in different tissues to achieve different biologic responses. Phospholipases $A_2$ relevant in inflammation are stimulated by such cytokines as TNF-α, GM-CSF, and IFN-γ, such growth factors as EGF, and the MAPK–PKC cascade. Glucocorticoids inhibit overall phospholipase $A_2$ activity by inducing the synthesis of **lipocortins,** a family of phospholipase $A_2$-inhibiting proteins (see below).

## CYCLOOXYGENASE PATHWAY

Unesterified intracellular arachidonic acid is immediately metabolized by either **cyclooxygenase, lipoxygenase,** or a cytochrome-containing **epoxygenase** enzyme. *The cyclooxygenase pathway leads to the formation of **prostaglandins, prostacyclin,** and **thromboxanes;** the lipoxygenase pathway leads to **leukotrienes** and **lipoxins;** and the epoxygenase pathway leads to epoxyeicosatetraenoic acids* (Fig. 40-1). Cyclooxygenases (also known as prostaglandin H synthases) are glycosylated, homodimeric, membrane-bound, heme-containing enzymes that are ubiquitous in animal cells from invertebrates to humans. *Two cyclooxygenase isoforms, denoted **COX-1** and **COX-2,** are found in humans.* Although COX-1 and COX-2 share 60% sequence homology and near superimposable three-dimensional structures, the genes are found on different chromosomes, and the enzymes differ in cellular, genetic, physiologic, pathologic, and pharmacologic profiles (Table 40-1). Cyclooxygenases catalyze two sequential reactions: (1) cyclooxygenase activity catalyzes the oxygen-dependent cyclization of arachidonic acid to prostaglandin $G_2$ ($PGG_2$); and (2) peroxidase activity catalyzes the reduction of $PGG_2$ to $PGH_2$.

*As a result of differences in cellular localization, regulatory profile, tissue expression, and substrate requirement, COX-1 and COX-2 ultimately produce different sets of eicosanoid products that are involved in two different pathways.* Constitutively expressed COX-1 is believed to function in physiologic, or "**housekeeping,**" activities such as vascular homeostasis, maintenance of renal and gastrointestinal blood flow, renal function, intestinal mucosal proliferation, platelet function, and antithrombogenesis. A number of "**as-needed,**" or specialized functions have been attributed to the inducible COX-2 enzyme, including roles in inflammation, fever, pain, transduction of painful stimuli in the spinal cord, mitogenesis (particularly in the gastrointestinal epithelium), renal adaptation to stresses, deposition of trabecular bone, ovulation, placentation, and uterine contractions of labor. The role of constitutive COX-2 expression in such areas of the nervous system as the hippocampus, hypothalamus, and amygdala remains to be elucidated.

Protein kinetic studies suggest that there may be a third functional cyclooxygenase isoform. The putative COX-3 isoform may be a product of the same gene as COX-1 but with different protein characteristics, possibly because of alternative mRNA splicing or post-translational modification. Furthermore, COX-3 may be a potential site of action of **acetaminophen.** However, definitive proof of the existence of COX-3 remains elusive.



Prostanoid backbone

**Figure 40-2. Prostanoid Structure.** The generic prostanoid structure is a theoretical 20-carbon carboxylic acid with a cyclopentane ring and a 15-hydroxyl group. All prostaglandins, thromboxanes, and prostacyclins are derived from this common structure.

## Prostaglandins

Prostaglandins are a large family of structurally similar compounds. The name of the family derives from their initial identification in the genitourinary system of male sheep. Prostaglandins all share a chemical structure, called a **prostanoid,** that is a 20-carbon carboxylic acid containing a cyclopentane ring and a 15-hydroxyl group (Fig. 40-2).

Prostaglandins are divided into three major subseries—$PG_1$, $PG_2$, and $PG_3$. The subscript numeral indicates the number of double bonds in the molecule. The $PG_2$ series is the most prevalent because these are direct derivatives of arachidonic acid, an eicosatetraenoic acid. The $PG_1$ series derive from the arachidonic acid precursor dihomo-$\gamma$-linolenic acid (DHGLA), an eicosatrienoic acid, while the $PG_3$ series derive from an eicosapentaenoic acid (EPA).

The prostaglandin $PGH_2$ represents the critical juncture of the cyclooxygenase pathway (see Fig. 40-3) because it is the precursor to $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, thromboxane $A_2$ ($TxA_2$), and prostacyclin ($PGI_2$). The distribution of these eicosanoids in various tissues is determined by the expression pattern of the different enzymes of prostaglandin synthesis in the tissues.

The prostaglandins are important in many physiologic processes, most of which are not directly related to inflammation. All of these functions are highlighted in Table 40-2. Note

**TABLE 40-1** Comparison of COX-1 and COX-2

| PROPERTY | COX-1 | COX-2 |
|---|---|---|
| Expression | Constitutive | Inducible; not normally present in most tissues<br>Constitutive in parts of nervous system |
| Tissue location | Ubiquitous expression | Inflamed and activated tissues |
| Cellular localization | Endoplasmic reticulum | ER and nuclear membrane |
| Substrate selectivity | Arachidonic acid, eicosapentaenoic acids | Arachidonic acid, $\gamma$-linolenate, $\alpha$-linolenate, linoleate, eicosapentaenoic acids |
| Role | Protection and maintenance functions | Proinflammatory and mitogenic functions |
| Induction | Generally no induction<br>hCG can upregulate COX-1 in amnion | Induced by LPS, TNF-$\alpha$, IL-1, IL-2, EGF, IFN-$\gamma$<br>mRNA rises 20–80-fold upon induction<br>Regulated within 1–3 hours |
| Inhibition | Pharmacologic: NSAIDs (low-dose aspirin) | In vivo: Anti-inflammatory glucocorticoids, IL-1$\beta$, IL-4, IL-10, IL-13<br>Pharmacologic: NSAIDs, COX-2 inhibitors |

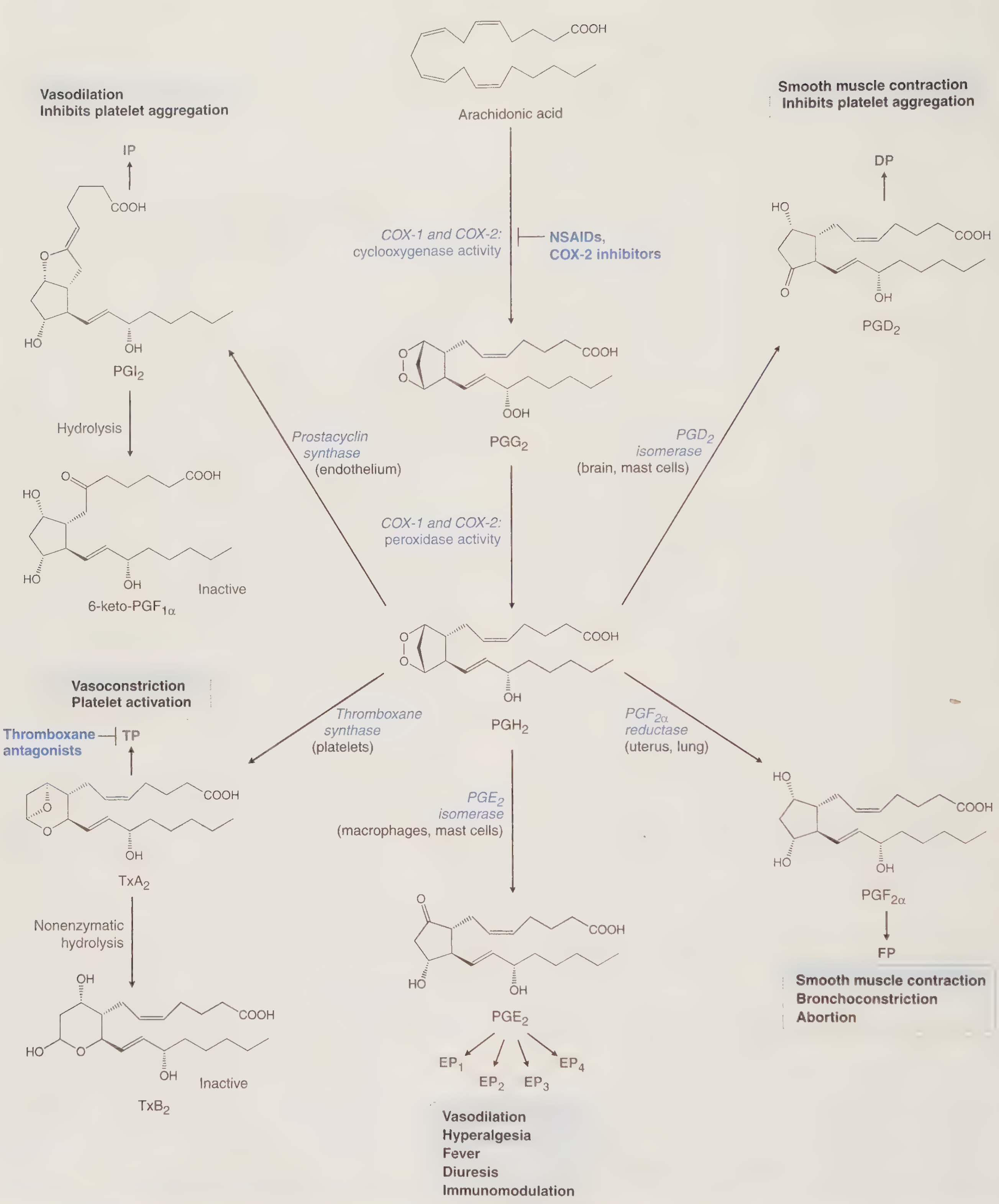

COOH
Arachidonic acid
COX-1 and COX-2:
cyclooxygenase activity
NSAIDs,
COX-2 inhibitors
O
O
COOH
OOH
$PGG_2$
COX-1 and COX-2:
peroxidase activity
O
O
COOH
OH
$PGH_2$
Vasodilation
Inhibits platelet aggregation
IP
COOH
O
HO
OH
$PGI_2$
Hydrolysis
O
COOH
HO
HO
OH
Inactive
6-keto-$PGF_{1\alpha}$
Prostacyclin
synthase
(endothelium)
Smooth muscle contraction
Inhibits platelet aggregation
DP
HO
COOH
O
OH
$PGD_2$
$PGD_2$
isomerase
(brain, mast cells)
Vasoconstriction
Platelet activation
Thromboxane
antagonists
TP
COOH
O
O
OH
$TxA_2$
Nonenzymatic
hydrolysis
OH
COOH
HO
O
OH
Inactive
$TxB_2$
Thromboxane
synthase
(platelets)
$PGE_2$
isomerase
(macrophages, mast cells)
O
COOH
HO
OH
$PGE_2$
$EP_1$
$EP_2$
$EP_3$
$EP_4$
Vasodilation
Hyperalgesia
Fever
Diuresis
Immunomodulation
$PGF_{2\alpha}$
reductase
(uterus, lung)
HO
COOH
HO
OH
$PGF_{2\alpha}$
FP
Smooth muscle contraction
Bronchoconstriction
Abortion

Figure 40-3. Prostaglandin Synthesis, Metabolism, Function, and Pharmacologic Inhibition. The biosynthetic pathways from arachidonic acid to prostaglandins, prostacyclin, and thromboxane are shown. Note that tissue-specific enzyme expression determines the tissues in which the various $PGH_2$ products are produced. NSAIDs and COX-2 inhibitors are the most important classes of drugs that modulate prostaglandin production. Thromboxane antagonists, although a promising pharmacologic strategy, do not currently have any clinical indications. COX, cyclooxygenase. PG, prostaglandin. Tx, thromboxane. DP, $PGD_2$ receptor. EP, $PGE_2$ receptor. FP, $PGF_{2\alpha}$ receptor. IP, $PGI_2$ receptor. TP, $TxA_2$ receptor. NSAID, nonsteroidal anti-inflammatory drug.

TABLE 40-2 Prostaglandin Products, Synthesis, Receptors, and Functions

| PROSTAGLANDIN | SYNTHETIC ENZYME | TISSUES EXPRESSING SYNTHETIC ENZYME | RECEPTOR TYPE AND SIGNALING MECHANISM | | FUNCTIONS |
|---|---|---|---|---|---|
| $PGD_2$ | $PGD_2$ isomerase | Mast cells<br>Neurons | DP | $G_s$ | Bronchoconstriction (asthma)<br>?Sleep control functions<br>?Alzheimer's disease |
| $PGE_2$ | $PGE_2$ isomerase | Many tissues, including macrophages and mast cells | EP1 | $G_q$ | ?Potentiation of responses to painful stimuli |
| | | | EP2 | $G_s$ | Vasodilation<br>Bronchoconstriction |
| | | | EP3 | $G_i$ | **Cytoprotective: modulates gastric mucosal acid secretion, mucus, and blood flow** |
| | | | EP4 | $G_s$ | Vasodilation<br>Bronchoconstriction |
| | | | Other | | **Inflammatory cell activation**<br>**Pyrexia**<br>Mucus production<br>?Possibly erectile function |
| $PGF_{2\alpha}$ | $PGF_{2\alpha}$ reductase | Vascular smooth muscle<br>Uterine smooth muscle | FP | $G_q$ | Vascular tone<br>Reproductive physiology (abortifacient)<br>Bronchoconstriction |

The prostanoid receptors are all G protein-coupled receptors.

especially the important housekeeping functions of $PGE_2$, broadly referred to as **cytoprotective** roles, in which such organs as gastric mucosa, myocardium, and renal parenchyma are shielded from the effects of ischemia by $PGE_2$-mediated vasodilation and regulation of blood flow. $PGE_2$ may also be involved in inflammatory cell activation, and $PGE_2$ that is synthesized by COX-2 and $PGE_2$ isomerase in cells near the hypothalamus may have a role in fever.

## Thromboxane and Prostacyclin

Platelets express high levels of the enzyme thromboxane synthase, but do not contain prostacyclin synthase. Therefore, *$TxA_2$ is the chief eicosanoid product of platelets.* $TxA_2$ has a half-life of only seconds before it is nonenzymatically hydrolyzed to inactive $TxB_2$. $TxA_2$, which signals via a 7-transmembrane G protein-coupled $G_q$ mechanism, is both a strong vasoconstrictor and a promoter of platelet adhesion and aggregation. In contrast, the vascular endothelium lacks thromboxane synthase but expresses prostacyclin synthase. *$PGI_2$ is, therefore, the chief eicosanoid product of the vascular endothelium.* $PGI_2$, which signals via $G_s$, functions as a vasodilator, venodilator, and inhibitor of platelet aggregation. In other words, $PGI_2$ is a physiologic antagonist of $TxA_2$. The vasodilatory actions of $PGI_2$, as with those of $PGE_2$, also confer cytoprotective properties.

*$TxA_2$ is a relatively stronger vasoconstrictor and platelet activator than $PGI_2$ is a vasodilator and platelet inhibitor. Therefore, the balance between $TxA_2$ and $PGI_2$ levels is critical in the regulation of systemic blood pressure and thrombogenesis.* Imbalances can lead to hypertension, ischemia, thrombosis, coagulopathy, myocardial infarction, and stroke. In certain populations of the Northern latitudes (including Inuit, Greenland, Irish, and Danish populations), the incidence of heart disease, stroke, and thromboembolic disorders is less than in Western populations. The diet of these Northern peoples is richer in whale and fish oils as compared to Western diets and, as a result, it contains relatively smaller amounts of arachidonic acid precursors but relatively larger amounts of EPAs, which are converted into $TxA_3$ and $PGI_3$ (Fig. 40-4A). Importantly, the vasoconstricting and platelet aggregating effects of $TxA_3$ are relatively weak as compared to the vasodilating and platelet inhibiting effects of $PGI_3$. As a result, the thromboxane–prostacyclin balance is tipped toward vasodilation, platelet inhibition, and antithrombogenesis, with a corresponding decline in the related diseases (Fig. 40-4B,C). This is one possible explanation for the observation that these populations have a lower incidence of heart disease and is a rationale for increasing dietary fish consumption in Western diets.

## LIPOXYGENASE PATHWAY

The lipoxygenase pathway represents the second major fate of arachidonic acid. This pathway leads to the formation of leukotrienes and lipoxins. Lipoxygenases are enzymes that catalyze the dioxygenation of arachidonic acid using nonheme

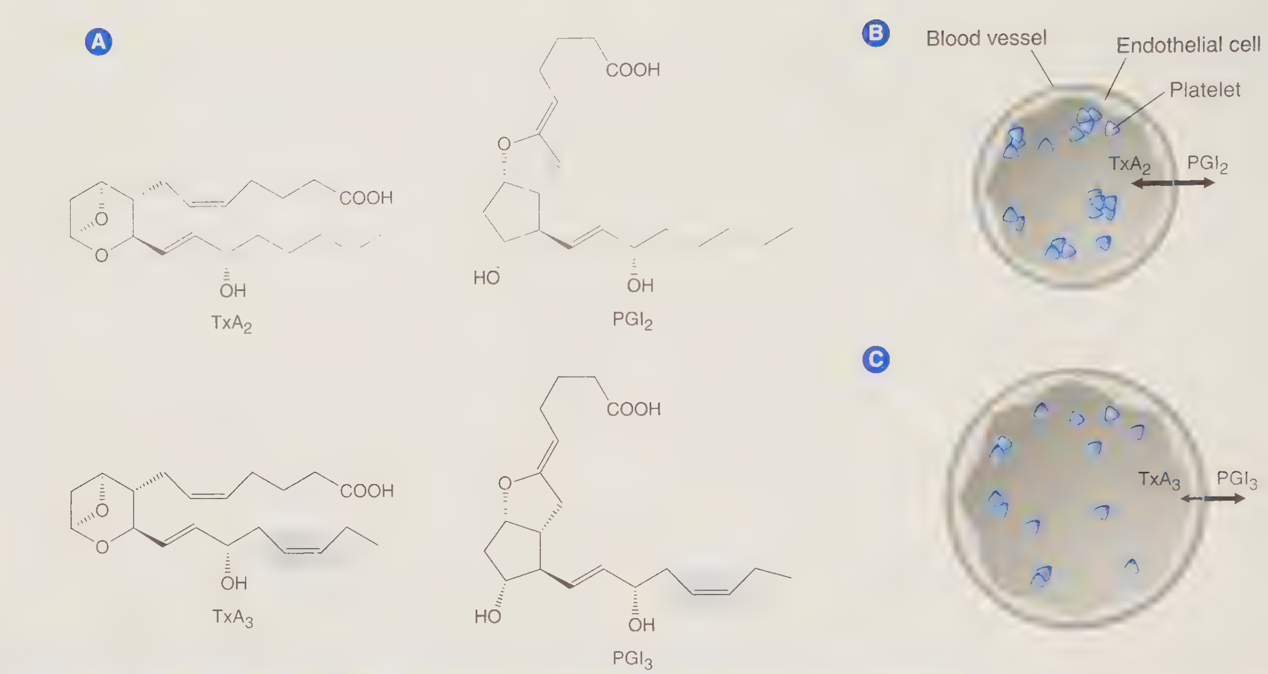


**Figure 40-4. Control of Vascular Tone and Platelet Activation by Thromboxanes and Prostacyclins.** **A.** In comparison to $TxA_2$, $TxA_3$ has a third double bond (highlighted in blue box) three carbons from the noncarboxylic acid end of the molecule (the "omega-3" position). By analogy, $PGI_3$ has an additional double bond (highlighted) compared to $PGI_2$. **B.** A cross section of a vessel is shown, with platelets in the vascular lumen. The arrows depict the relative balance between $TxA_2$-mediated vasoconstriction and $PGI_2$-mediated vasodilation. The figure also shows the relative balance between $TxA_2$-mediated platelet aggregation and $PGI_2$-mediated inhibition of platelet aggregation. $TxA_2$ is slightly more dominant than $PGI_2$, so there is net vasoconstriction and mild platelet aggregation. **C.** This figure shows the balance between thromboxane and prostacyclin action in an individual on a diet rich in fish oil (which contains high concentrations of omega-3 fatty acids). On this diet, there are relatively higher levels of $TxA_3$, which is considerably less potent than $TxA_2$, and $PGI_3$, which is approximately as potent as $PGI_2$. Therefore, the balance is shifted toward net vasodilation and net inhibition of platelet aggregation. This shift may lower the incidence of thrombogenic and ischemic diseases, such as myocardial infarction and stroke.

iron. Three lipoxygenases, 5-, 12-, and 15-lipoxygenases (5-LOX, etc.), are found in humans (Table 40-3). The lipoxygenases are named for the position on arachidonic acid on which they catalyze the insertion of molecular $O_2$. The immediate products of lipoxygenase reactions are hydroperoxyeicosatetraenoic acids (**HPETE**). HPETEs can be reduced to the corresponding hydroxyeicosatetraenoic acids (**HETE**) by enzymes using glutathione peroxidase (GSP). 5-HPETE formed by 5-LOX is the direct precursor to $LTA_4$, which itself is the direct precursor to all other leukotrienes (Fig. 40-5). Lipoxygenases are also involved in converting 15-HETE and $LTA_4$ to lipoxins.

5-LOX requires translocation to the nuclear membrane for activity. The protein 5-lipoxygenase-activating protein (**FLAP**) helps 5-LOX translocate to the nuclear membrane, form an active enzyme complex, and accept the arachidonic acid substrate from phospholipase $A_2$.

## Leukotrienes

Leukotriene synthesis begins with the 5-LOX-mediated conversion of 5-HPETE to leukotriene $A_4$ ($LTA_4$). Therefore, *5-LOX catalyzes the first two steps in leukotriene biosynthesis* (Fig. 40-5). It is not known whether the 5-HPETE diffuses out of the 5-LOX enzymatic active site between these steps, or remains bound to the same 5-LOX enzyme for both reactions.

$LTA_4$ is converted to either $LTB_4$ or $LTC_4$. The enzyme $LTA_4$ hydrolase converts $LTA_4$ to $LTB_4$ in neutrophils and erythrocytes. Conversion to $LTC_4$ occurs in mast cells, eosinophils, basophils, and macrophages by the addition of a $\gamma$-glutamylcysteinylglycine tripeptide (glutathione) to $LTA_4$. $LTC_4$, $LTD_4$, $LTE_4$, and $LTF_4$, which represent the **cysteinyl leukotrienes,** are interconverted by addition and removal of amino acid portions of the $\gamma$-glutamylcysteinylglycine tripeptide (Fig. 40-5).

$LTB_4$ acts via two G protein-coupled receptors, BLTR1 and BLTR2. Binding of $LTB_4$ to BLTR1, which is expressed mainly on tissues involved in inflammation (leukocytes, thymus, spleen), leads to proinflammatory sequelae, most importantly neutrophil chemotaxis, aggregation, and transmigration through epithelium and endothelium. $LTB_4$ may upregulate lysosomal function and free radical production, enhance cytokine production, and potentiate the action of natural killer cells. The role of the ubiquitously expressed BLTR2 remains unknown.

The cysteinyl leukotrienes bind to CysLT1 receptors to cause vasoconstriction, bronchospasm, and increased vascular permeability. Cysteinyl leukotrienes are responsible for the inflammation, hyper-reactivity to stimuli, and airway and vascular smooth muscle contraction that occur in asthma, psoriasis, and various arthritic, allergic, and hypersensitivity processes.

## Lipoxins

Lipoxins (**lipox**ygenase **in**teraction products) are derivatives of arachidonic acid containing four conjugated double bonds and three hydroxyl groups. The two lipoxins, $LXA_4$ and $LXB_4$ (Fig. 40-6), are believed to modulate the effects of leukotrienes.

**TABLE 40-3** Tissue Expression of Lipoxygenases and Products of Lipoxygenase Action

| LIPOXYGENASE | TISSUE EXPRESSION | PRODUCTS | PATHWAYS | NOTES |
|---|---|---|---|---|
| 5-LOX | Neutrophils | 5-HPETE / 5-HETE | Leukotrienes / Lipoxins | Requires FLAP for activity |
| | Macrophages | $LTA_4$ | Lipoxins | |
| | Mast cells | Epoxytetraene | Lipoxins / Aspirin-triggered lipoxins | |
| | Eosinophils | | | |
| 12-LOX | | | | |
| Platelet type | Platelets | 12-HPETE / 12-HETE | Lipoxins | |
| | Megakaryocytes | Epoxytetraene | | |
| | (Tumors) | | | |
| Epidermal type | Skin | | | |
| Leukocyte type | Macrophages | | | |
| | GI system | | | |
| | Brain | | | |
| 15-LOX | Macrophages | 15-HPETE / 15-HETE | Lipoxins | |
| | Monocytes | Epoxytetraene | Lipoxins | |
| | Airway epithelium | | | |

FLAP, 5-lipoxygenase-activating protein. LOX, lipoxygenase.

At sites of inflammation, there is typically an inverse relationship between the amounts of lipoxin and leukotriene present. This finding has led to the suggestion that lipoxins may act as negative regulators of leukotriene action. $LXA_4$ receptors have been identified in the lung, spleen, and blood vessels, and on neutrophils. Lipoxins have been shown to inhibit neutrophil chemotaxis, adhesion, and transmigration through endothelium (by decreasing P-selectin expression), inhibit eosinophil recruitment, stimulate vasodilation (by inducing synthesis of $PGI_2$ and $PGE_2$), inhibit $LTC_4$- and $LTD_4$-stimulated vasoconstriction, inhibit $LTB_4$ inflammatory effects, and inhibit the function of NK cells. *Lipoxin production may, therefore, be important in the resolution of inflammation, and an imbalance in lipoxin–leukotriene homeostasis may be a key factor in the pathogenesis of inflammatory disease.* For example, it is possible that Mrs. D.'s chronic joint inflammation involves an imbalance in the relative amounts of leukotrienes and lipoxins in her joints.

## EPOXYGENASE PATHWAY

Microsomal cytochrome P450 epoxygenases are able to oxidize arachidonic acid, resulting in the formation of epoxyeicosatetraenoic acid (EET) and hydroxyacid derivatives (Fig. 40-1). The epoxygenation of arachidonic acid results in four different EETs, depending on which double bond in arachidonic acid is modified. Dihydroxy derivatives of EETs, formed by hydrolysis, may have roles in the regulation of smooth muscle cells and vascular tone by inhibiting the $Na^+/K^+$-ATPase, or in renal function by regulating ion absorption and secretion. With respect to inflammation, dihydroxy EET derivatives inhibit platelet cyclooxygenase and inhibit the expression of intercellular adhesion molecules (ICAMs). Downregulation of ICAMs inhibits platelet and inflammatory cell aggregation. Thus, *as with lipoxins, EETs may play a role in regulating inflammation at certain sites and within select tissues.* Future research may reveal more definitive functions for EETs in inflammation.

## ISOPROSTANES

Phospholipid-esterified arachidonic acid is susceptible to free-radical-mediated peroxidation; cleavage of these modified lipids from the phospholipid by phospholipase $A_2$ gives rise to the isoprostanes (Fig. 40-1). Isoprostanes are found in the blood at levels much higher than those of cyclooxygenase products. Two isoprostanes in particular, 8-epi-$PGF_{2\alpha}$ and 8-epi-$PGE_2$, are potent vasoconstrictors. Isoprostanes may function in activating NF-$\kappa$B, phospholipase C$\gamma$, protein kinase C, and calcium flux. *Because the rate of formation of isoprostanes depends on cellular oxidation conditions, isoprostane levels may be indicative of oxidative stress.* Urinary isoprostane concentrations have been used as a marker of oxidative stress in ischemic syndromes, reperfusion injury, atherosclerosis, and hepatic diseases. Isoprostanes are not, however, known to have any role in inflammation.

## METABOLIC INACTIVATION OF EICOSANOIDS

Prostaglandins, leukotrienes, thromboxanes, and lipoxins are degraded by hydroxylation, $\beta$-oxidation (resulting in a loss of two carbons), or $\omega$-oxidation (to dicarboxylic acid derivatives). These degradation processes render the molecules more hydrophilic and, therefore, more excretable in the urine.

## INTEGRATED INFLAMMATION SCHEMA

As described in the above discussion, eicosanoids are synthesized in numerous complex reactions. It is not necessary to remember every mediator, but rather to understand the

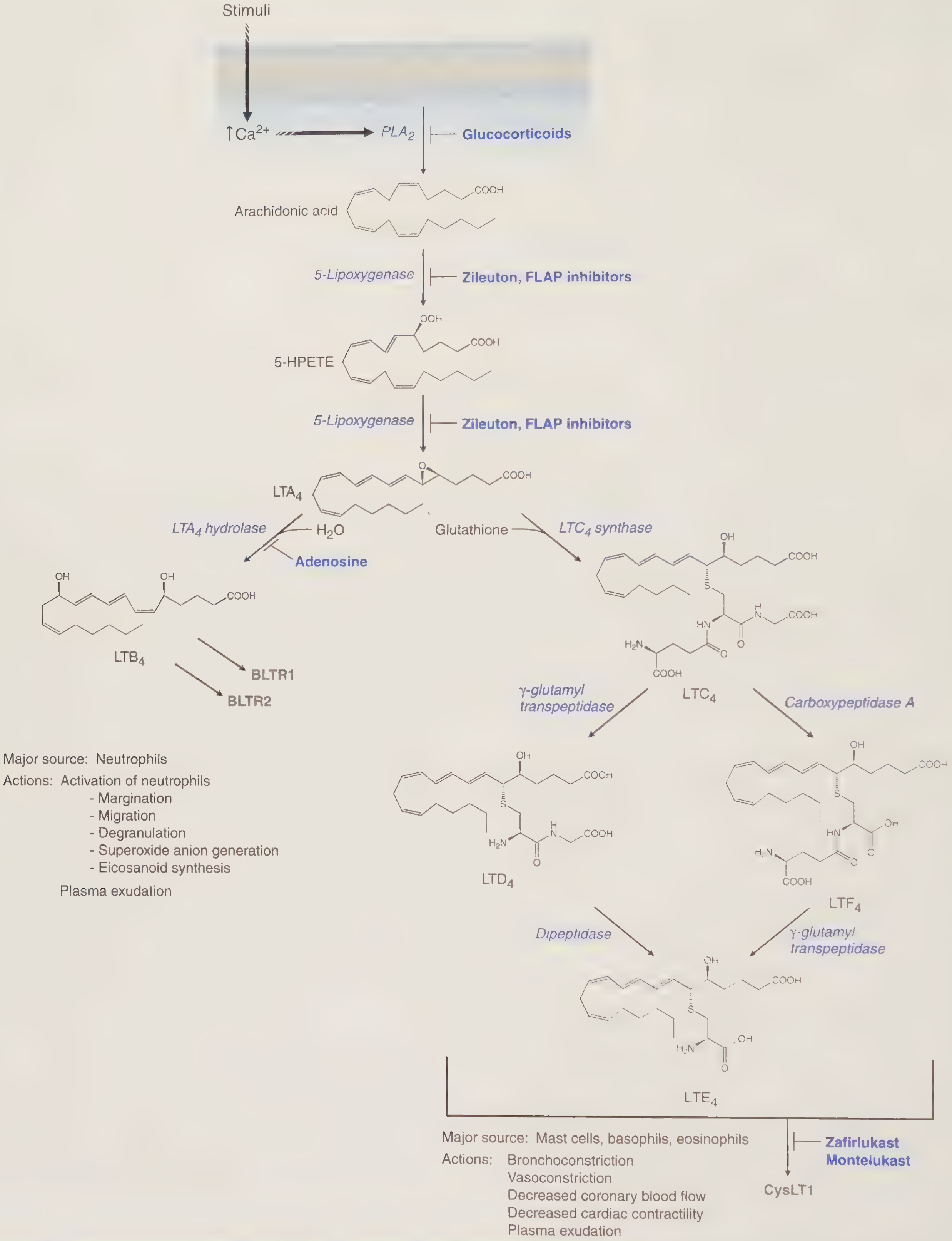

Stimuli
↑Ca2+
PLA2
Glucocorticoids
Arachidonic acid
COOH
5-Lipoxygenase
Zileuton, FLAP inhibitors
OOH
COOH
5-HPETE
5-Lipoxygenase
Zileuton, FLAP inhibitors
O
COOH
LTA4
LTA4 hydrolase
H2O
Adenosine
Glutathione
LTC4 synthase
OH
OH
COOH
LTB4
BLTR1
BLTR2
Major source: Neutrophils
Actions: Activation of neutrophils
- Margination
- Migration
- Degranulation
- Superoxide anion generation
- Eicosanoid synthesis
Plasma exudation
OH
COOH
S
H N
COOH
HN
H2N
O
O
COOH
LTC4
γ-glutamyl transpeptidase
Carboxypeptidase A
OH
COOH
S
H2N
H N
COOH
O
LTD4
OH
COOH
S
HN
OH
O
H2N
O
COOH
LTF4
Dipeptidase
γ-glutamyl transpeptidase
OH
COOH
S
H2N
OH
O
LTE4
Major source: Mast cells, basophils, eosinophils
Actions: Bronchoconstriction
Vasoconstriction
Decreased coronary blood flow
Decreased cardiac contractility
Plasma exudation
Zafirlukast
Montelukast
CysLT1

**Figure 40-5. Leukotriene Synthesis, Metabolism, Function, and Pharmacologic Inhibition.** The biosynthetic pathways from arachidonic acid to the leukotrienes are shown. Glucocorticoids decrease phospholipase $A_2$ ($PLA_2$) activity, thereby preventing the synthesis of all leukotrienes (LTs). Zileuton and 5-lipoxygenase-activating protein (FLAP) inhibitors prevent the conversion of arachidonic acid to 5-HPETE and $LTA_4$; zileuton is used in the chronic management of asthma. Adenosine has been shown to inhibit $LTB_4$ synthesis in neutrophils, although adenosine is not used pharmacologically for this purpose. Zafirlukast and montelukast are antagonists at CysLT1, the receptor for all cysteinyl leukotrienes; these drugs are used in the chronic management of asthma. BLTR1 and BLTR2, $LTB_4$ receptors. CysLT1, receptor for $LTC_4$, $LTD_4$, $LTE_4$, and $LTF_4$.

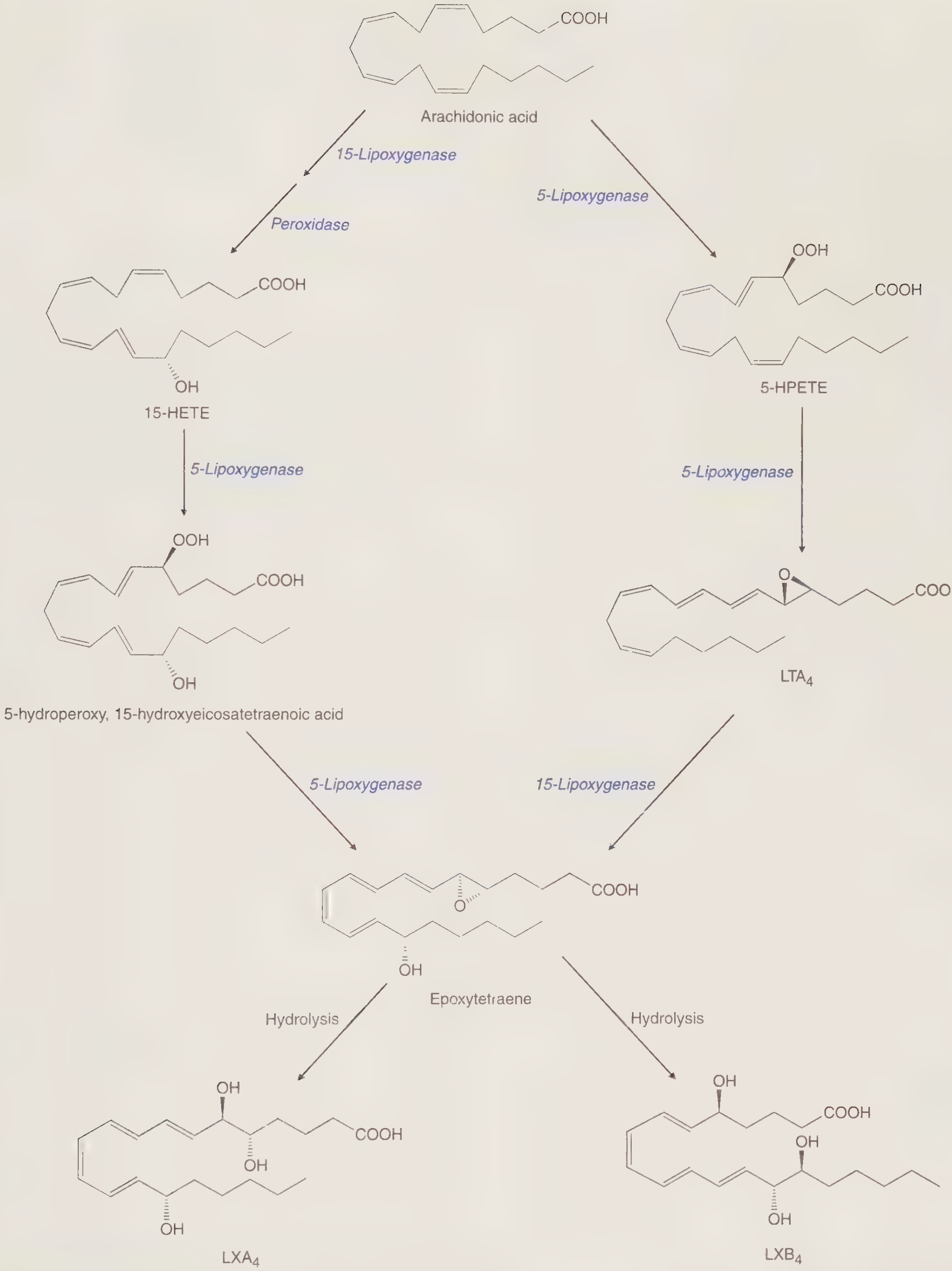

COOH
Arachidonic acid
15-Lipoxygenase
5-Lipoxygenase
Peroxidase
OOH
COOH
COOH
OH
15-HETE
5-HPETE
5-Lipoxygenase
5-Lipoxygenase
OOH
COOH
O
COOH
OH
LTA4
5-hydroperoxy, 15-hydroxyeicosatetraenoic acid
5-Lipoxygenase
15-Lipoxygenase
COOH
O
OH
Epoxytetraene
Hydrolysis
Hydrolysis
OH
COOH
OH
OH
OH
COOH
OH
OH
LXA4
LXB4

**Figure 40-6. Lipoxin Synthesis.** Two parallel routes lead to biosynthesis of the lipoxins. In each pathway, three sequential lipoxygenase reactions are required, followed by hydrolysis. The immediate precursor of the lipoxins is epoxytetraene; hydrolysis of epoxytetraene yields the lipoxins. Left pathway: Arachidonic acid is converted to 15-HETE by sequential activity of 15-lipoxygenase and peroxidase. 15-HETE is converted by 5-lipoxygenase to the chemical intermediate 5-hydroperoxy, 15-hydroxyeicosatetraenoic acid, and 5-lipoxygenase acts on this intermediate to form epoxytetraene. Right pathway: Arachidonic acid is converted to 5-HPETE by 5-lipoxygenase, and 5-HPETE is converted to $LTA_4$ by further action of 5-lipoxygenase. $LTA_4$ is converted to epoxytetraene by 15-lipoxygenase. Common pathway: Epoxytetraene is hydrolyzed to the active lipoxins $LXA_4$ and $LXB_4$. The lipoxins are thought to have an anti-inflammatory role, because they appear to be negative regulators of leukotriene action.

general scheme of these synthetic pathways. This section, along with Table 40-4, provides a concise overview of the physiologic functions of eicosanoids relevant to inflammation.

Acute inflammation is the result of an intricate network of molecular and cellular interactions induced by responses to a variety of stimuli, such as trauma, ischemia, infectious agents, or antibody reactions. Acute superficial inflammation generates local pain, edema, erythema, and heat; inflammation in visceral organs can have similar symptoms and result in severe impairment of organ function.

*Leukotrienes and lipoxins, as well as thromboxanes, prostaglandins, and prostacyclins, are critical for generating and mediating inflammatory responses.* The inflammatory cascade is initiated when cells in a particular region are exposed to a foreign substance or are damaged. That insult stimulates a local cytokine cascade (including interleukins or TNF) that raises the expression of COX-2 mRNA and enzyme levels. COX-2 then facilitates production of the proinflammatory and chemotactic eicosanoids.

Locally high concentrations of $PGE_2$, $PGI_2$, and cysteinyl leukotrienes promote the accumulation and infiltration of inflammatory cells by increasing blood flow and vascular permeability. $LTB_4$ and 5-HETE are also important in attracting and activating neutrophils and platelets. $LTB_4$, formed by activated neutrophils at the site of inflammation, recruits and activates additional neutrophils and lymphocytes so that these cells adhere to endothelial surfaces and transmigrate into the interstitial spaces. Increased vascular permeability also results in fluid leak and cellular infiltration, causing edema.

With the aggregation of a multitude of inflammatory cells, **transcellular biosynthetic routes** are exploited to generate eicosanoids (Fig. 40-7). *In transcellular synthesis, eicosanoid intermediates are donated from one cell type to another to generate a larger diversity of eicosanoids.* This demonstrates the importance of cellular adhesion and cell–cell interaction in inflammatory and immune responses.

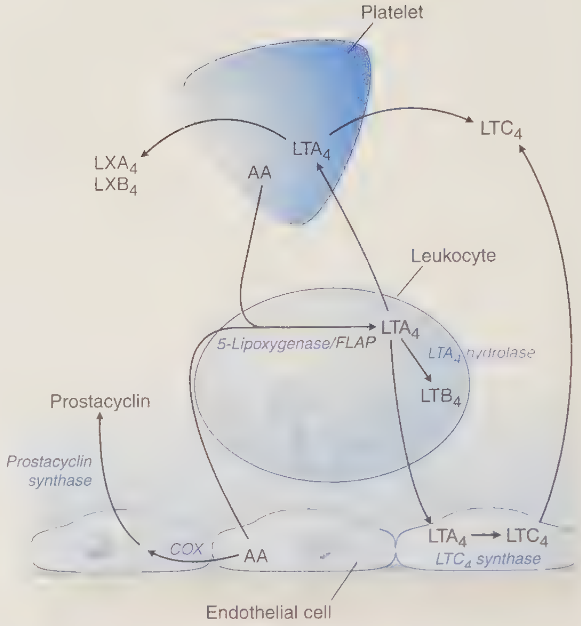


**Figure 40-7. Examples of Transcellular Biosynthesis.** Transcellular biosynthesis is used to synthesize lipoxins and cysteinyl leukotrienes. In the example shown here, the leukocyte (neutrophil) obtains arachidonic acid (AA) from endothelial cells and from platelets, and uses this AA to synthesize leukotriene $A_4$ ($LTA_4$) and leukotriene $B_4$ ($LTB_4$). Leukotriene $A_4$ is transferred from the leukocyte to platelets and endothelial cells, which synthesize and secrete leukotriene $C_4$ ($LTC_4$). Platelets also synthesize lipoxins ($LXA_4$, $LXB_4$) from leukotriene $A_4$, and the endothelial cells synthesize prostacyclin using AA from endogenous stores. Note that the eicosanoids synthesized within each cell type are determined by the enzymatic repertoire of that cell type: for example, neutrophils synthesize primarily $LTA_4$ and $LTB_4$ because they express 5-lipoxygenase and $LTA_4$ hydrolase, whereas endothelial cells synthesize prostacyclin and $LTC_4$ because they express prostacyclin synthase and $LTC_4$ synthase.

The body tries to ensure that the inflammatory response cannot proceed unchecked. Lipoxins are believed to help resolve inflammation. COX-2-derived eicosanoids may also function in wound healing and resolution. $PGE_2$ has been found to inhibit the function of B and T lymphocytes and NK cells, while $LTB_4$ and the cysteinyl leukotrienes inhibit T lymphocyte proliferation. These factors may coordinately prevent the transition from acute to chronic inflammation.

**TABLE 40-4** Roles of Eicosanoids in the Steps of Inflammation

| ACTION | EICOSANOIDS INVOLVED |
|---|---|
| Vasoconstriction | $PGF_{2\alpha}$, $TxA_2$, $LTC_4$, $LTD_4$, $LTE_4$ |
| Vasodilation (erythema) | $PGI_2$, $PGE_1$, $PGE_2$, $PGD_2$, $LXA_4$, $LXB_4$, $LTB_4$ |
| Edema (swelling) | $PGE_2$, $LTB_4$, $LTC_4$, $LTD_4$, $LTE_4$ |
| Chemotaxis, leukocyte adhesion | $LTB_4$, HETE, $LXA_4$, $LXB_4$ |
| Increased vascular permeability | $LTC_4$, $LTD_4$, $LTE_4$ |
| Pain and hyperalgesia | $PGE_2$, $PGI_2$, $LTB_4$ |
| Local heat and systemic fever | $PGE_2$, $PGI_2$, $LXA_4$ |

## PATHOPHYSIOLOGY

The immune response and subsequent inflammation are the body's mechanisms for fighting foreign invaders. This response is designed to remove the inciting stimulus and resolve tissue damage. In some cases, the response mechanism itself causes local tissue damage, such as when activated neutrophils release proteases and reactive oxygen species. In other settings, if the inflammatory reactions persist for too long or the immune system misidentifies a part of self as foreign; these abnormal, misdirected immune responses can cause significant and chronic injury.

Selected inflammatory diseases in which eicosanoids are implicated, including asthma, inflammatory bowel disease, rheumatoid arthritis, and glomerulonephritis, are profiled below. Other diseases not discussed here, but that have a possible eicosanoid-related inflammatory basis, include atherogenesis, myocardial infarction, certain skin disorders, reperfusion injuries, Alzheimer's disease, and adult respiratory distress syndrome.

## ASTHMA

Asthma is a chronic inflammatory disorder typified by intermittent attacks of dyspnea, coughing, and wheezing. Symptoms result from chronic airway inflammation, hyperreactivity, constriction, and obstruction. In asthma, the response to antigens in the lungs initiates cytokine cascades leading to generation of $PGD_2$ and leukotrienes. Elaboration of $LTB_4$ attracts inflammatory cells and promotes cellular aggregation. $LTB_4$ acts particularly on B lymphocytes to cause activation, proliferation, and differentiation. $LTB_4$ also promotes expression of FCεRII receptors (receptors for the constant chain of IgE antibodies) on mast cells and basophils; these receptors bind IgE that is released by antigen-stimulated B lymphocytes. $LTC_4$ and $LTD_4$ are extremely potent bronchoconstrictive compounds, being over 1,000 times more potent than histamine. These cysteinyl leukotrienes also cause the airway epithelium to secrete mucus, while impairing the clearance of this mucus by inhibiting the beating of cilia on airway epithelium. Mucus secretion is exacerbated by neutrophils and eosinophils, which become part of the inflammatory exudate clogging the airways. $LTD_4$ and $LTE_4$ recruit eosinophils to asthmatic airways; eosinophils integrate signals from T lymphocytes and, when activated, release factors that damage the airway epithelium.

In a mouse model of asthma in which the 5-LOX gene was knocked out, no airway hyperresponsiveness or eosinophilia was observed. This result underscores the important role of the leukotriene products of 5-LOX activity in the pathogenesis of asthma. The role of leukotriene inhibitors in asthma treatment is discussed below; for additional information, refer to Chapter 45, Integrative Inflammation Pharmacology: Asthma.

## INFLAMMATORY BOWEL DISEASE

Inflammatory bowel diseases, which include Crohn's disease and ulcerative colitis, are two idiopathic, chronic, relapsing, ulcerative diseases of the gastrointestinal tract. Although physiologically and pathologically distinct, elevated $LTB_4$ production in the affected mucosa results in abnormal leukocyte infiltration into the parenchyma in both conditions. Chronic inflammation and leukocyte infiltration leads to progressive mucosal damage, with overt histologic changes. Crohn's disease is characterized by focal damage, fissuring ulcers, and granulomas, while mucosal inflammation and colonic dilatation are found in ulcerative colitis. Both diseases increase the risk of adenocarcinoma of the colon in the affected areas.

## RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic, systemic autoimmune and inflammatory disease that primarily attacks the joints, but also affects the skin, cardiovascular system, lungs, and muscles. Rheumatoid arthritis affects up to 1.5% of North Americans and is three times more prevalent in females than in males. Autoimmune targeting of normal joint proteins results in inflammation, with local release of cytokines, TNF, growth factors, and interleukins, all of which induce COX-2 expression. *Levels of COX-2 enzyme and $PGE_2$ have been found to be markedly elevated in the synovial fluid of affected joints.* Other COX-2-derived eicosanoids activate the surrounding endothelium to recruit inflammatory cells. Macrophages elaborate collagenase and proteases, while lymphocytes cause immune complex formation; both processes further damage joint tissue and provide a substrate for chronic inflammation. Common findings include synovitis, leukocytosis, rheumatoid nodules, and the presence of rheumatoid factor (a circulating antibody directed against IgG).

As an older Caucasian woman, Mrs. D. is in a risk group for rheumatoid arthritis. Autoimmune destruction of her joints resulted in the findings of a high erythrocyte sedimentation rate and a low hematocrit, both of which indicate a state of chronic inflammation, synovial leukocytosis, and the progressive loss of joint mobility and function. For additional information on rheumatoid arthritis, refer to Chapter 43, Pharmacology of Immunosuppression.

## GLOMERULONEPHRITIS

Glomerulonephritis represents a large group of inflammatory renal conditions that ultimately lead to renal failure through deterioration of renal hemodynamics and glomerular filtration. Local complement activation promotes neutrophil and macrophage infiltration. Infiltration of the glomerulus is a characteristic early pathologic finding that correlates with abnormal levels of $LTB_4$, which is synthesized by $LTA_4$ hydrolase located in the kidney mesangium. $LTB_4$ is a substrate for the synthesis of $LTC_4$ and $LTD_4$, which facilitate neutrophil adhesion to the glomerular mesangium and epithelium. All of the cysteinyl leukotrienes ($LTC_4$, $LTD_4$, $LTE_4$, and $LTF_4$) promote endothelial and mesangial proliferation. Cysteinyl leukotrienes also directly affect glomerular function; specifically, $LTC_4$ and $LTD_4$ decrease renal blood flow and GFR by vasoconstricting arterioles and contracting mesangial spaces. Studies with inhibitors have confirmed the roles of leukotrienes in glomerulonephritis. LOX inhibitors administered at early stages of glomerulonephritis prevent glomerular inflammation and evidence of structural damage. Both LOX inhibitors and $LTD_4$ receptor antagonists improve GFR and decrease proteinuria.

Interestingly, the kidney mesangium expresses both $LTA_4$ hydrolase and 12-LOX, conferring the ability to synthesize either $LTB_4$ or $LXA_4$. At low concentrations, $LTA_4$ is used primarily for $LTB_4$ formation; these conditions correspond to the initiation of inflammation or to a low level of chronic inflammation. Conversely, when $LTA_4$ concentrations are relatively high, as in long-standing inflammation, $LTA_4$ is converted mostly into $LXA_4$, which provides an autoinhibitory effect on the inflammatory response. In the glomerulus, $LXA_4$ serves to counteract the deleterious pro-inflammatory consequences of leukotrienes, as well as the effect of leukotrienes on GFR, in part by raising afferent arteriole flow via vasodilation.

## CANCER

Long-term epidemiologic studies have shown a correlation between chronic NSAID therapy and decreased incidence of colorectal cancer. Human colorectal adenomas and carcinomas have been found to express abundant COX-2 but not COX-1; similar results have been obtained in gastric adenocarcinomas and breast tumors. In these tissues, COX-2 is believed to generate $PGE_2$ and other eicosanoids that promote tumor growth. The perinuclear localization of the COX-2 enzyme (Table 40-1) has suggested the potential for an intracellular function of eicosanoid products in oncogenesis. Some eicosanoid derivatives have been shown to bind to homologs of the retinoic acid receptor (RXR) family of transcription factors, which are involved in many functions including the regulation of cell growth and differentiation. Overexpression of COX-2 would generate eicosanoids that could flood RXR signaling pathways and provide excessive growth stimuli. A COX-2 inhibitor has been approved as a therapy for patients at risk for one form of cancer, familial adenomatous polyposis (see discussion of COX-2 inhibitors below).

# PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic intervention in eicosanoid metabolism is particularly suited to controlling inflammation because of the extensive and diverse roles of the many eicosanoids, the complexity of the pathways offering a variety of targets, and the goal of inhibition (as opposed to upregulation) of the inflammation and immune responses. Pharmacologic interventions can be directed at any of a number of steps outlined above to achieve the desired effects and specificities. Strategies considered here include altering the expression of key enzymes, competitively and noncompetitively inhibiting the activity of enzymes, activating receptors with exogenous receptor agonists, and preventing receptor activation with exogenous receptor antagonists. As always, the potential therapeutic benefits must be weighed against the potential side effects.

## PHOSPHOLIPASE INHIBITORS

Inhibition of phospholipase $A_2$ prevents the generation of arachidonic acid, the rate-limiting step in eicosanoid synthesis. In the absence of pro-inflammatory mediators derived from arachidonic acid, inflammation is limited.

**Glucocorticoids** (also known as corticosteroids, of which **prednisone** is a member) are a mainstay of therapy in a multitude of autoimmune and inflammatory diseases. Glucocorticoids induce a family of secreted calcium- and phospholipid-dependent proteins called **lipocortins.** Lipocortin inhibits the action of phospholipase $A_2$, and, thereby, limits the release of arachidonic acid.

Small molecule inhibitors of specific phospholipases are in development; these drugs may offer the potential for decreased unwanted side effects associated with glucocorticoid use (for a discussion of the effects of glucocorticoids, see Chapter 26, Pharmacology of the Adrenal Cortex).

## CYCLOOXYGENASE INHIBITORS

A number of cyclooxygenase pathway inhibitors exist; these agents are some of the most frequently prescribed drugs in medicine. The nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are the most commonly used agents in this class.

### Traditional Nonselective Inhibitors: NSAIDs

*NSAIDs are important because of their combined anti-inflammatory, antipyretic, and analgesic activity.* The ultimate goal of most NSAID therapies is to inhibit COX-2 competitively and, thereby, to prevent the generation of pro-inflammatory eicosanoids and to limit the extent of inflammation, fever, and pain. The drugs' antipyretic activity may be related to their decreasing the levels of $PGE_2$, particularly in the region of the brain surrounding the hypothalamus. *Despite the benefits of NSAIDs, these drugs only suppress the signs of the underlying inflammatory response.*

A multitude of NSAIDs have been developed over the last century; most are polycyclic carboxylic acid derivatives. All NSAIDs, except aspirin, act as reversible, competitive inhibitors of cyclooxygenase. These drugs block the hydrophobic channel in cyclooxygenase in which the substrate arachidonic acid binds, thereby preventing access of arachidonic acid to the active site of the enzyme. Traditional NSAIDs inhibit both COX-1 and COX-2 to different degrees. Because of inhibition of COX-1, long-term NSAID therapy has many deleterious effects. The cytoprotective roles of the COX-1 eicosanoid products are eliminated, leading to a spectrum of **NSAID-induced gastropathy** including dyspepsia, gastrotoxicity, subepithelial damage and hemorrhage, gastric mucosal erosion, frank ulceration, and gastric mucosal necrosis. (Recall Mrs. D.'s case. Patients with gastric hemorrhage have bleeding into the stomach, where the digestion of hemoglobin produces a compound that, when regurgitated, has the color and consistency of coffee-grounds.) Regulation of blood flow to the kidney is likewise perturbed, decreasing GFR and potentially causing renal ischemia, papillary necrosis, interstitial nephritis, and renal failure. Epidemiologic studies have estimated that 20–30% of hospitalizations of patients over the age of 60 are due to complications of NSAID use.

The organic acid functionality of NSAIDs confers important pharmacokinetic properties on these drugs, including near-complete absorption from the gut, binding to plasma albumin, accumulation in cells at the site of inflammation, and efficient renal excretion. NSAIDs can be divided into short (< 6 hours) and long (> 10 hours) half-life classes. NSAIDs with long elimination half-lives include naproxen, salicylate, piroxicam, and phenylbutazone.

Chemical classification of the NSAIDs is based on the structure of a key moiety in each subclass of drugs (Fig. 40-8). The following discussion groups the NSAIDs by chemical class; a discussion of the choice of a particular NSAID for a given clinical situation follows the descriptions of the individual agents.

#### *Salicylates*

Salicylates include **aspirin** (acetylsalicylic acid) and its derivatives. Aspirin is used to treat mild to moderate pain,

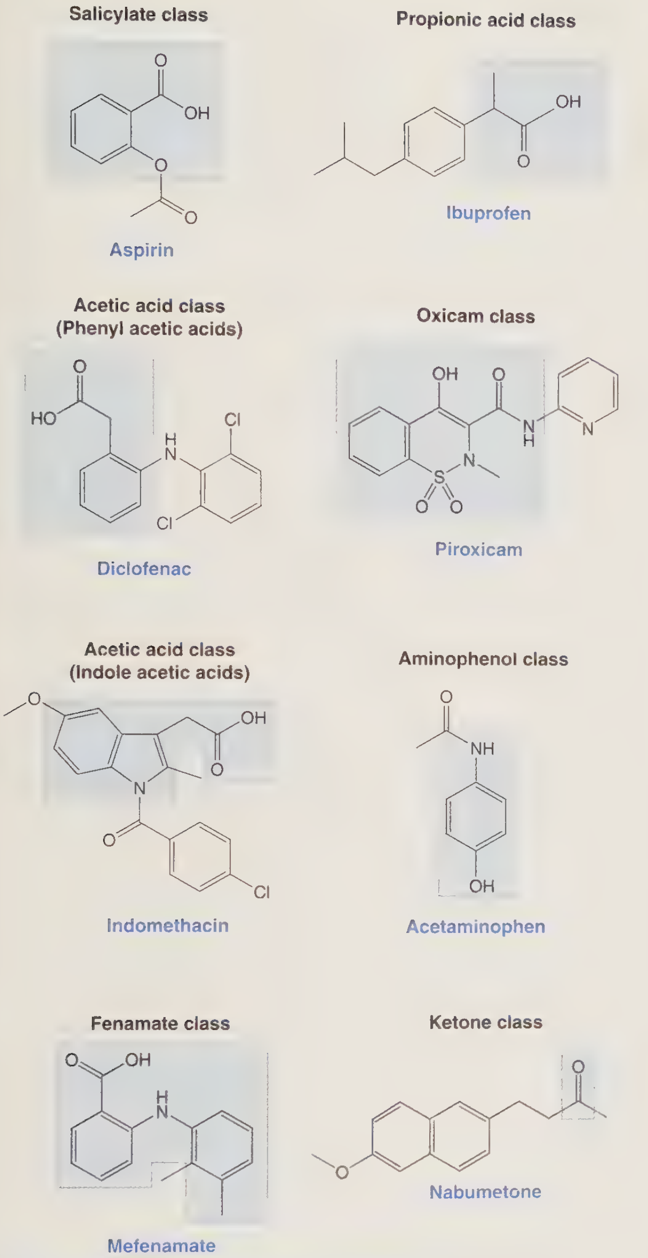


**Figure 40-8. Structural Classes of NSAIDs.** Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally hydrophobic molecules, most of which have a carboxylic acid group. NSAIDs are categorized by classes depending on one or more of the key moieties in the structure. The moiety that is common to members of each class is highlighted by a box. The structure helps to determine the pharmacokinetic properties of each particular NSAID. Note that acetaminophen is not actually an NSAID, because it has only weak anti-inflammatory properties; this drug is included here because, as with NSAIDs, acetaminophen is commonly used for its analgesic and antipyretic effects.

headache, myalgia, and arthralgia. *In contrast to other NSAIDs, aspirin acts in an irreversible manner by acetylating the active site serine residue in both COX-1 and COX-2.* Acetylation of COX-1 destroys the enzyme's cyclooxygenase activity and, thereby, prevents the formation of COX-1-derived prostaglandins, thromboxanes, and prostacyclin. Salicylates (along with indomethacin, piroxicam, and ibuprofen) may also inhibit the neutrophil oxidative burst by reducing NADPH oxidase activity.

*Daily low-dose aspirin is used as an antithrombogenic agent for prophylaxis and postevent management of myocardial infarction and stroke.* Recall that aspirin is antithrombogenic because of its irreversible inhibition of COX, which prevents platelets from synthesizing $TxA_2$. Within an hour of oral aspirin administration, the COX-1 activity in existing platelets is irreversibly destroyed. The importance of this lies in the fact that platelets, being anucleate, circulate in the bloodstream for 10 days but cannot synthesize new protein. Therefore, the irreversibly acetylated COX-1 molecules cannot be replaced by freshly synthesized proteins, and these platelets are irreversibly inhibited for the lifetime of the platelets. Although aspirin also irreversibly inhibits endothelial cell COX-1, the endothelial cells can synthesize new COX-1 protein and, thus, can resume synthesis of $PGI_2$. *A single administration of aspirin decreases for several days the amount of thromboxane that can be generated, shifting the $TxA_2$–$PGI_2$ balance toward $PGI_2$-mediated vasodilation, platelet inhibition, and antithrombogenesis.*

Aspirin-mediated inhibition of COX-2 prevents the generation of normal prostaglandin metabolites. Unlike COX-1, which is totally inactivated, aspirin-modified COX-2 retains a part of its catalytic activity and can form a new product, 15-(R)-HETE, from arachidonic acid. By analogy to normal lipoxin synthesis (Fig. 40-6), 5-LOX then converts 15-(R)-HETE to 15-epi-lipoxins, which are relatively stable stereoisomers of lipoxins that are collectively called **aspirin-triggered lipoxins (ATLs)**. *15-Epi-lipoxins mimic the functions of lipoxins as anti-inflammatory agents.* 15-Epi-lipoxins may represent an endogenous mechanism of anti-inflammation, and their production is believed to mediate at least part of the anti-inflammatory effects of aspirin. Development of 15-epi-lipoxin analogues could lead to an anti-inflammatory drug that does not have the side effects associated with COX-1 inhibition.

Aspirin is generally well tolerated. Its major toxicities are the gastropathy and nephropathy common to all NSAIDs. Long-term aspirin therapy can lead to gastrointestinal ulceration and hemorrhage, nephrotoxicity, and hepatic injury. Two unique toxicities are **aspirin-induced airway hyperreactivity** in asthmatics and **Reye's syndrome.** The prevalence of aspirin sensitivity among patients with asthma is estimated to be approximately 10%. Exposure to aspirin in these patients leads to ocular and nasal congestion and severe airway obstruction. Aspirin-sensitive patients are also reactive to some other NSAIDs, including indomethacin, naproxen, ibuprofen, mefenamate, and phenylbutazone. One possible etiology of aspirin/NSAID sensitivity in asthmatics is that exposure to these drugs leads to increased levels of leukotrienes, which are implicated in the pathogenesis of asthma. Because NSAIDs inhibit COX-1, excess arachidonic acid that would otherwise have been used to synthesize prostaglandins is instead available for shunting to the lipoxygenase pathway to form leukotrienes (see Fig. 40-1).

Reye's syndrome is a condition characterized by hepatic encephalopathy and liver steatosis in young children. Aspirin therapy in children during the course of a febrile viral infection has been implicated as a potential etiology of the liver damage. Although a causal link between aspirin and Reye's syndrome has not been definitively established, aspirin is generally not administered to children because of the fear of Reye's syndrome.

### Propionic Acid Derivatives

Propionic acid NSAIDs include **ibuprofen, naproxen, ketoprofen,** and **flurbiprofen.** Ibuprofen is a relatively potent analgesic used in rheumatoid arthritis (as in the case of Mrs. D., to relieve intermittent pain), osteoarthritis, ankylosing spondylitis, gout, and primary dysmenorrhea. Naproxen has a long serum half-life, is twenty times more potent than aspirin, directly inhibits leukocyte function, and causes less severe gastrointestinal side effects as compared with aspirin.

### Acetic Acid Derivatives

Acetic acid NSAIDs include the indole acetic acids—**indomethacin, sulindac,** and **etodolac**—and the phenylacetic acids **diclofenac** and **ketorolac** (a substituted phenylacetic acid derivative). Besides inhibiting cyclooxygenase, many of the acetic acid NSAIDs promote the incorporation of unesterified arachidonic acid into triglyceride, thus reducing the availability of the substrate for cyclooxygenase. Indomethacin is a direct inhibitor of neutrophil motility, but it is not tolerated by patients as well as ibuprofen. Diclofenac is a more potent anti-inflammatory than indomethacin and naproxen. Diclofenac also reduces intracellular arachidonic acid concentrations by altering cellular fatty acid transport. Ketorolac is primarily employed for its strong analgesic properties, particularly in postsurgical patients.

The acetic acid NSAIDs are mostly used to relieve symptoms in the long-term treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and other musculoskeletal disorders. Use of acetic acid NSAIDs causes gastrointestinal ulceration and, rarely, hepatitis and jaundice. Indomethacin also has a specific use in promoting the closure of a patent ductus arteriosus in newborns; this effect may be due to the drug's inhibition of the synthesis of the vasodilatory eicosanoids $PGE_2$ and $PGI_2$.

### Oxicam Derivatives

The efficacy of **piroxicam** is similar to that of aspirin, naproxen, and ibuprofen in the treatment of rheumatoid arthritis and osteoarthritis, but, as compared to these drugs, piroxicam may be better tolerated. Piroxicam has additional effects in the modulation of neutrophil function by inhibiting collagenase, proteoglycanase, and the oxidative burst. Because of its extremely long half-life, piroxicam can be administered once daily. As with other NSAIDs, piroxicam displays gastrointestinal side effects such as ulceration, and it prolongs bleeding time.

### Fenamate Derivatives

The two fenamate NSAIDs are **mefenamate** and **meclofenamate.** Both inhibit cyclooxygenases but also antagonize prostanoid receptors to various degrees. Because fenamates have less anti-inflammatory activity and are more toxic than aspirin, there is little advantage to their use. Mefenamate is used only for primary dysmenorrhea, whereas meclofenamate is used in the treatment of rheumatoid arthritis and osteoarthritis.

### Ketones

**Nabumetone** is a ketone prodrug that is oxidized in vivo to the active acid form. Compared to other nonselective NSAIDs, nabumetone has preferential activity against COX-2. The incidence of gastrointestinal side effects is relatively low, although headache and dizziness are frequently reported.

## Acetaminophen

**Acetaminophen,** although sometimes classified with the NSAIDs, is technically not an NSAID. *Although acetaminophen has analgesic and antipyretic effects similar to aspirin, the anti-inflammatory effect of acetaminophen is insignificant because of its weak inhibition of cyclooxygenase-2.* Acetaminophen therapy can, however, be valuable in patients who are sensitive to the side effects of aspirin. The most important side effect of acetaminophen is hepatotoxicity. Modification of acetaminophen by hepatic cytochrome P450 enzymes produces a reactive molecule, which is detoxified by conjugation with glutathione. An overdose of acetaminophen can overwhelm glutathione stores, leading to cellular and oxidative damage and, in severe cases, to acute hepatic necrosis (see Chapter 4, Drug Metabolism).

## Selection of the Appropriate NSAID

The anti-inflammatory, analgesic, and antipyretic effects of the NSAIDs do vary among the many agents in this class. However, despite the drugs' differences in chemistry, tissue selectivity, enzyme selectivity, pharmacokinetics, and pharmacodynamics, these differences in efficacy may not be clinically significant. The choice of NSAID does not generally make a substantial difference in treating rheumatoid arthritis or osteoarthritis. *Designing NSAID therapy is considered more of an art than a science, and therapy should be directed at achieving the desired anti-inflammatory, analgesic, and antipyretic effects while minimizing the side-effects due to COX-1 inhibition.* The gastric side effects of long-term NSAID therapy can be reduced by coadministration of H2 receptor antagonists or proton pump inhibitors (refer to Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease).

## COX-2 Inhibitors

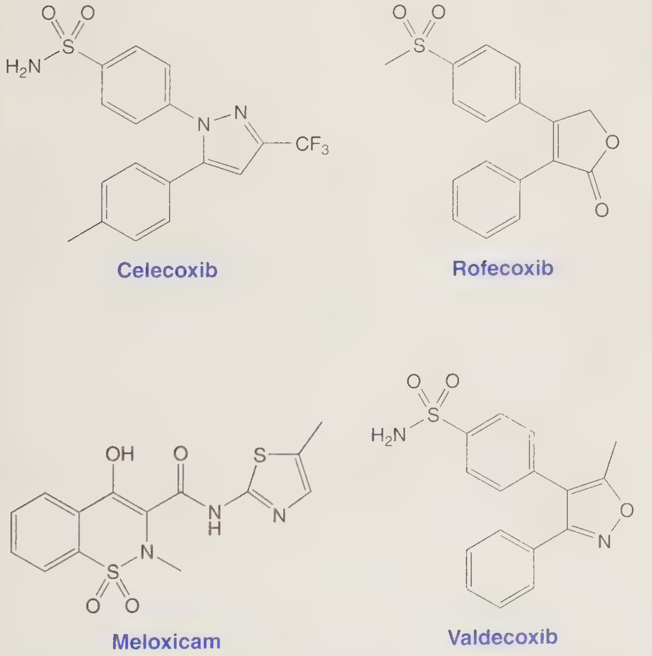
Because of the severe gastrointestinal side effects associated with long-term NSAID therapy, recent strategies for inhibition of cyclooxygenase pathways have focused on selective inhibition of COX-2. This approach should have the theoretical advantage of inhibiting the chemical mediators responsible for inflammation, while maintaining the cytoprotective effects of the products of COX-1 activity.

### COX-2 Selective Inhibitors

Although COX-2 was identified only in the 1990s, intense research has already led to the development of COX-2 selective inhibitors. *Compared with COX-1, COX-2 has a larger hydrophobic channel through which substrate (arachidonic acid) enters the active site.* This structural difference has allowed the development of drugs that bind with higher affinity to COX-2 than to COX-1.

The approved COX-2 selective inhibitors **celecoxib, rofecoxib, valdecoxib,** and **meloxicam** (Fig. 40-9) are sulfonic acid derivatives that display 100-fold selectivity for COX-2 compared to COX-1. The relative inhibition of the two cyclooxygenase isozymes by these drugs in any given tissue is also a function of drug metabolism, pharmacokinetics, and, possibly, enzyme polymorphisms. The COX-2 selective inhibitors have the same anti-inflammatory, antipyretic, and analgesic benefits as the NSAIDs, but are safer with respect to gastrointestinal side effects. Note that Mrs. D. took advantage of the improved safety profile of COX-2 selective inhibitors when she switched from ibuprofen to a COX-2 inhibitor, in part, because of symptomatic and endoscopic evidence of NSAID-induced gastropathy. The long-term safety profiles of COX-2 inhibitors are unknown. There is some concern that COX-2 inhibitors—in particular, rofecoxib—have deleterious effects on the cardiovascular and renal systems by inducing hypertension, renal failure, and cardiac failure. In addition, inhibition of COX-2 may generate potential problems in wound healing and angiogenesis. Finally, COX-2 selective inhibitors are much more expensive than equivalent doses of many NSAIDs, especially aspirin.

Celecoxib is approved for osteoarthritis, rheumatoid arthritis, acute pain in adults, and primary dysmenorrhea. This drug is also approved to reduce the number of adenomatous colorectal polyps in individuals with **familial adenomatous polyposis.** Celecoxib decreases the activity of PPARδ, a transcription factor that heterodimerizes with the RXR transcription factors that are involved in growth regulation. It is not yet clear whether COX-2 inhibitors bind directly to PPARδ or whether they lead indirectly to the synthesis of other molecules that inhibit PPARδ. Nevertheless, inhibition of PPARδ prevents signaling through the PPARδ pathway and, thus, removes a potent mitogenic stimulus that could function in the development of colon cancer.



Celecoxib

Rofecoxib

Meloxicam

Valdecoxib

**Figure 40-9. COX-2 Selective Inhibitors.** COX-2 selective inhibitors are hydrophobic sulfonic acid derivatives. As with traditional NSAIDs, these molecules block the hydrophobic channel leading to the active site of cyclooxygenase, and thus inhibit the enzyme. However, the COX-2 selective inhibitors are generally larger molecules than NSAIDs and, therefore, preferentially inhibit COX-2 compared to COX-1 because the hydrophobic channel of COX-2 is larger. (That is, COX-2 selective inhibitors are too bulky to access the smaller hydrophobic channel of the COX-1 enzyme.) The COX-2 selective inhibitors display approximately 100-fold greater selectivity for COX-2 as compared to COX-1.

Valdecoxib is approved for osteoarthritis, rheumatoid arthritis, and primary dysmenorrhea. Rofecoxib is approved for osteoarthritis, acute pain in adults, and primary dysmenorrhea. Meloxicam is approved only for osteoarthritis.

The second-generation COX-2 inhibitors in development—such as parecoxib (a prodrug of valdecoxib), etoricoxib, and lumiracoxib—are expected to demonstrate increased selectivity for COX-2 over COX-1, and to eliminate any potential cardiovascular side effects of the existing COX-2 inhibitors.

### *Glucocorticoids*

Prednisone, and other glucocorticoids administered at pharmacologic doses, inhibit the action of COX-2 by several mechanisms: (1) repressing COX-2 gene expression; (2) repressing the expression of cytokines that activate COX-2; and (3) limiting the available pool of COX-2 substrate (arachidonic acid) by inhibiting phospholipase $A_2$. In combination, these mechanisms create a powerful anti-inflammatory effect because virtually all eicosanoid pathways are inhibited. Because of this profound and global suppression of immune and inflammatory responses, glucocorticoids are indicated in the treatment of a number of autoimmune conditions (see Chapter 43, Pharmacology of Immunosuppression).

### *Cytokine Inhibitors*

The proinflammatory cytokines TNF-α and IL-1 both upregulate COX-2. Novel molecular technologies have provided the ability to inhibit the action of these cytokines and, thus, to inhibit the process whereby an injurious stimulus activates COX-2 and initiates the inflammatory response. Three TNF-α antagonists, **etanercept, infliximab,** and **adalimumab,** are currently used in the treatment of rheumatoid arthritis. Etanercept consists of the extracellular domain of the TNF-α receptor coupled to human IgG1; infliximab is a humanized mouse monoclonal antibody directed against TNF-α; and adalimumab is a fully humanized monoclonal IgG1 antibody directed against TNF-α. With few side effects, these drugs halt joint destruction and bone erosion, decrease pain, improve swollen and tender joints, and limit overall disease progression in rheumatoid arthritis. Infliximab has also been approved for the treatment of Crohn's disease. **Anakinra** is a recombinant form of the human IL-1 receptor produced in *E. coli;* this drug is approved for use in rheumatoid arthritis. Additional IL-1 antagonists are being developed for use in inflammatory and autoimmune diseases. For more information on these agents, refer to Chapter 43.

## PROSTANOID RECEPTOR MIMETICS

Several interesting applications for prostanoid receptor agonists are listed in the Drug Summary Table at the end of this chapter.

## THROMBOXANE ANTAGONISTS

Both $TxA_2$ receptor antagonists and thromboxane synthase inhibitors could represent extremely powerful and selective agents capable of inhibiting platelet activity and protecting against thrombosis and vascular disease. These thromboxane antagonists could theoretically serve as "super aspirin" platelet inhibitors in the management of patients with cardiovascular disease. $TxA_2$ receptor antagonists, unlike aspirin, would also be expected to block the vasoconstrictive action of the isoprostanes. Such compounds as **dazoxiben** and **pirmagrel** inhibit thromboxane synthase, whereas **ridogrel** is a $TxA_2$ receptor antagonist. These thromboxane antagonists have not yet found clinical utility, however, because the clinical benefit of these drugs is not significantly greater than that of aspirin, which is far less expensive.

## LEUKOTRIENE INHIBITION

### Lipoxygenase Inhibition

Inhibition of the lipoxygenases has the potential to represent a major therapeutic modality in diseases involving leukotriene-mediated pathophysiology, including asthma, inflammatory bowel disease, and rheumatoid arthritis. Lipoxygenase inhibition is an attractive therapeutic approach in these diseases because leukotrienes are thought not to have significant roles in physiology aside from inflammation.

Several strategies are possible for the design of lipoxygenase inhibitors, based on the structure, function, and mechanism of the lipoxygenase enzymes. Suicide inhibitors of lipoxygenase (e.g., derivatives of arachidonic acid with triple bonds instead of double bonds), which become covalently bound to the active site and render it inactive, have been developed but are not available for clinical use. Radical scavengers such as catechols, BHT (butylated hydroxytoluene), and $\alpha$-tocopherol trap the radical intermediates in the lipoxygenase reaction and, thereby, prevent the functioning of the enzyme, but these nonspecific compounds cannot be used clinically for lipoxygenase inhibition.

Drugs that impair or alter the ability of lipoxygenase to utilize its nonheme iron properly would be expected to inhibit the activity of the enzyme. The only iron chelator drug in clinical use is **zileuton** (Fig. 40-10A), a benzothiophene derivative of N-hydroxyurea that inhibits 5-LOX. In asthma, zileuton induces bronchodilation, improves symptoms, and generates long-lasting improvement in pulmonary function tests. Zileuton is effective in the treatment of asthma induced by cold, drugs, and allergens. Because of its low bioavailability, low potency, and significant side effects such as liver toxicity, zileuton is not as widely used as the other anti-leukotriene asthma drugs (see below).

### 5-Lipoxygenase-Activating Protein (FLAP) Inhibition

Interfering with the role of FLAP could represent an approach to the selective inhibition of 5-LOX activity and leukotriene function. Recall that 5-LOX is activated after the enzyme localizes to the nuclear membrane and docks with FLAP; FLAP also binds arachidonic acid released by phospholipase $A_2$ and shuttles it to the 5-LOX active site. FLAP inhibitors have been developed that both prevent and reverse LOX binding to FLAP and block the arachidonic acid binding site, but no FLAP inhibitor compounds are available for clinical use.

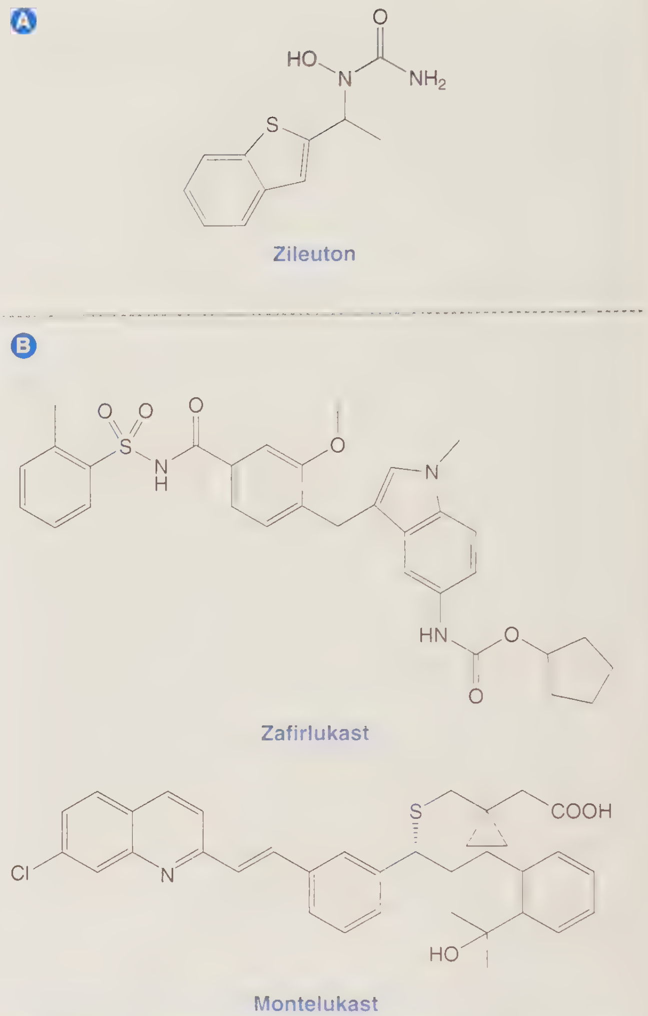


Figure 40-10. Leukotriene Pathway Inhibitors. **A.** Zileuton is a 5-lipoxygenase inhibitor that blocks the synthesis of leukotrienes from arachidonic acid. **B.** Zafirlukast and montelukast are leukotriene receptor antagonists. All three drugs are approved for the prevention and chronic treatment of asthma in both adults and children. None of these drugs, however, are effective in the treatment of acute asthma attacks.

### Leukotriene Synthesis Inhibitors

Other than zileuton (see above), no specific inhibitors of the enzymes involved in leukotriene synthesis are currently available for clinical use. **Adenosine** has been shown to inhibit $LTB_4$ synthesis in neutrophils, possibly by interfering with the influx of calcium. Furthermore, adenosine is thought to have a role in limiting cell and tissue injury during inflammation. High cell turnover at inflammatory sites generates high local concentrations of adenosine, which may decrease $LTB_4$ synthesis and reduce leukocyte recruitment and activation. Adenosine has not yet been developed for pharmacologic use in the control of inflammation, however.

### Leukotriene Receptor Antagonists

Leukotriene receptor antagonism represents the final mechanism for inhibiting leukotriene-mediated inflammation. Cysteinyl leukotriene receptor (CysLT1) antagonists are effective against antigen-, exercise-, cold-, and aspirin-induced asthma. These agents significantly improve bronchial tone, pulmonary function tests, and asthma symptoms. **Montelukast** and **zafirlukast** (Fig. 40-10B) are the currently available cysteinyl leukotriene receptor antagonists; the main clinical application for these antagonists is in the treatment of asthma.

More potent CysLT1 antagonists are under development, including pobilukast, tomelukast, and verlukast. Further research will likely elaborate cysteinyl leukotriene receptor subtypes and their respective tissue distributions, which could offer the possibility of tissue-targeted antagonism and the application of these tissue-selective antagonists to such other conditions as rheumatoid arthritis, inflammatory bowel disease, and various allergic disorders.

## LIPOXINS AND ASPIRIN-TRIGGERED LIPOXINS

Lipoxins and aspirin-triggered lipoxins (ATLs) offer the potential to antagonize the inflammatory actions of leukotrienes and other inflammatory mediators, and to promote resolution on inflammation. Because lipoxins have no other known roles in physiology, they are expected to have selective actions with few side effects. Stable analogues of lipoxins and ATLs are currently being developed as novel agonists to enhance the resolution of acute inflammation.

## *Conclusion and Future Directions*

Eicosanoids are critical mediators of homeostasis and of many pathophysiologic processes, especially inflammation. Arachidonic acid, the primary substrate, is converted into prostaglandins, thromboxanes, prostacyclin, leukotrienes, lipoxins, isoprostanes, and epoxyeicosatetraenoic acids. Prostaglandins have diverse roles in vascular tone regulation, gastrointestinal regulation, uterine physiology, analgesia, and inflammation. Prostacyclin and thromboxane coordinately control vascular tone, platelet activation, and thrombogenesis. Leukotrienes are the chief inflammatory mediators of leukocyte chemotaxis, infiltration, and activation. Lipoxins antagonize the effects of leukotrienes and reduce the extent of inflammation.

Pharmacologic interventions at many critical points in these pathways are useful in limiting inflammatory sequelae. Glucocorticoids inhibit several steps in eicosanoid generation, including the rate-determining step involving phospholipase $A_2$. However, chronic glucocorticoid use is associated with deleterious side effects, including osteoporosis, muscle wasting, and abnormal carbohydrate metabolism. Cyclooxygenase inhibitors block the first step of prostanoid synthesis and prevent the generation of prostanoid mediators of inflammation. Lipoxygenase inhibitors, FLAP inhibitors, leukotriene synthesis inhibitors, and leukotriene receptor antagonists prevent leukotriene signaling, thereby limiting inflammation and its deleterious effects. Future drug development efforts will allow selective targeting of eicosanoid pathways involved in many clinical conditions.

## *Suggested Readings*

Brink C. Dahlen SE, Drazen J, et al. International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. Pharmacol Rev 2003;55:195–227. (*International consensus report on eicosanoid receptors and their antagonists.*)

Dahlen SE, Haeggstrom JZ, Samuelsson B, et al. Leukotrienes as targets for treatment of asthma and other diseases: current basic and clinical research. Am J Respir Crit Care Med 2000;161:S1. (*Highlights the potential applications of leukotriene pathway inhibitors.*)

FitzGerald GA. COX-2 and beyond: approaches to prostaglandin inhibition in human disease. Nat Rev Drug Discov 2003;2:879–890. (*Structural and biochemical basis for differences in COX-1 and COX inhibition.*)

Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001;294:1871–1875. (*Advances in the biochemistry of eicosanoid pathways.*)

Samuelsson B, Dahlen SE, Lindgren JA, et al. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science 1987;237:1171–1176. (*Review of the discovery and actions of leukotrienes and lipoxins.*)

Serhan CN, Oliw E. Unorthodox routes to prostanoid formation: new twists in cyclooxygenase-initiated pathways. J Clin Invest 2001;107:1481–1489. (*Review of the anti-inflammatory actions of lipoxins and the discovery of resolvins and related compounds.*)

Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Ann Rev Pharmacol Toxiciol 1998;38:97–120. (*Historical overview of prostaglandin research, including discussion of the pharmacologic manipulation of these pathways.*)

**Drug Summary Table** **Pharmacology of Eicosanoids**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **NSAIDs**<br>*Mechanism—Inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)* | | | | |
| **Aspirin (salicylate class)** | Pain, headache, platelet inhibition for stroke / MI prophylaxis | All NSAIDs can cause:<br>NSAID-induced gastropathy: risk of GI bleeding, ulceration, perforation<br>NSAID-nephropathy | Aspirin hypersensitivity, aspirin-triggered asthma, Reye's syndrome | |
| **Ibuprofen (propionic acid class)** | Osteoarthritis, rheumatoid arthritis, dysmenorrhea, gout | | | |
| **Indomethacin (acetic acid class)** | Rheumatoid arthritis, osteoarthritis, close PDA (see notes) | | | Used to close patent ductus arteriosus. Compare this to action of prostanoid mimetics, which can be used to maintain patency of ductus arteriosus. |
| **Ketorolac (acetic acid class)** | Rheumatoid arthritis, osteoarthritis | | | Analgesia in postsurgical patients |
| **Piroxicam (oxicam class)** | Rheumatoid arthritis, osteoarthritis | | | Long half-life; once daily dosing |
| **Meclofenamate (fenamate class)** | Rheumatoid arthritis, osteoarthritis, primary dysmenorrhea | | | |
| **Nabumetone (ketone class)** | Rheumatoid arthritis, osteoarthritis | | | Greatest selectivity for COX-2 of all NSAIDs |
| **ACETAMINOPHEN**<br>*Mechanism—See Notes* | | | | |
| **Acetaminophen** | Analgesia, antipyretic | Hepatotoxic | | Generally safe for use in patients undergoing surgery and dental procedures<br>May inhibit COX-3 isoform in CNS<br>Antidote for acetaminophen overdose is N-acetylcysteine |
| **COXIBS (COX-2 SELECTIVE INHIBITORS)**<br>*Mechanism—Inhibit COX-2* | | | | |
| **Celecoxib** | Osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, acute pain in adults, familial adenomatous polyposis | Dyspepsia and abdominal pain | Hypersensitivity or allergy to NSAIDs or sulfa drugs | All coxibs decrease efficacy of ACE inhibitors<br>For all coxibs, incidence of gastropathy and nephropathy may be less than that associated with NSAIDs |
| **Valdecoxib** | Osteoarthritis, rheumatoid arthritis, primary dysmenorrhea | | | |
| **Rofecoxib** | Osteoarthritis, primary dysmenorrhea, acute pain in adults | GI bleeding, ulceration and perforation; renal papillary necrosis | Hypersensitivity or reaction to other NSAIDs. Monitor dosing when coadministered with P450 inducer. | |
| **Meloxicam** | Osteoarthritis | GI bleeding, ulceration and perforation | Hypersensitivity or allergy to NSAIDs | |

| | | | | |
|---|---|---|---|---|
| **GLUCOCORTICOIDS**<br>*Mechanism—Inhibit COX-2 expression; induce lipocortins, which inhibit phospholipase $A_2$* | | | | |
| **Glucocorticoids** | Chronic asthma, rheumatoid arthritis, etc. See Chapter 43. | Immunosuppression | See Chapter 43. | See Chapter 26, Pharmacology of the Adrenal Cortex, and Chapter 43, Pharmacology of Immunosuppression |
| **CYTOKINE ANTAGONISTS**<br>*Mechanism—Inhibit TNF-α* | | | | |
| **Etanercept** | Rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis | Immunosuppression | Hypersensitivity and sepsis | See Chapter 43 |
| **Infliximab** | Rheumatoid arthritis, Crohn's disease | Immunosuppression | Hypersensitivity to murine products | See Chapter 43 |
| **Adalimumab** | Rheumatoid arthritis | Immunosuppression | Hypersensitivity and sepsis | See Chapter 43 |
| *Mechanism—Inhibit IL-1* | | | | |
| **Anakinra** | Rheumatoid arthritis | Immunosuppression | Hypersensitivity | See Chapter 43 |
| **PROSTANOID MIMETICS**<br>*Mechanism—Prostanoid receptor agonists* | | | | |
| **$PGD_2$ analogues** | Glaucoma and myometrial hyperactivity | | | |
| **Alprostadil** | Maintenance of patent ductus arteriosus or treatment of erectile dysfunction | | | $PGE_1$ analogue with vasodilator properties. Maintaining patent ductus arteriosus beneficial in tetralogy of Fallot, Eisenmenger pulmonary hypertension, and aortic valve atresia. |
| **Misoprostol** | Cytoprotective effects in long-term NSAID therapy; abortifacient with mifepristone | | | $PGE_1$ analogue with vasodilator properties. Also used in peptic ulcer disease (see Chapter 44). |
| **Other $PGE_2$ analogues** | Cytoprotective effects in long-term NSAID therapy | | | Vasodilator |
| **$PGF_{2\alpha}$ analogues (Carboprost)** | Abortifacient; contraception | | | Stimulates uterine contraction for abortifacient activity; luteolytic activity controls fertility |
| **Latanoprost**<br>**Bimatoprost**<br>**Travoprost** | Glaucoma | | | $PGF_{2\alpha}$ analogues with vasodilator properties; ocular hypotensive agents |
| **Epoprostenol** | Pulmonary hypertension, vascular diseases | | | Prostacyclin analogue with vasodilator properties |

(Continued)

**Drug Summary Table** **Pharmacology of Eicosanoids** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **THROMBOXANE ANTAGONISTS (NOT USED CLINICALLY)**<br>*Mechanism—Inhibit thromboxane synthase or block thromboxane receptor* | | | | |
| **Dazoxiben**<br>**Pirmagrel**<br>**Ridogrel** | No clinical uses established | | | Advantages of these agents over aspirin are unproven. Little effect on platelet aggregation. |
| **LOX INHIBITORS**<br>*Mechanism—Inhibit 5-LOX* | | | | |
| **Zileuton** | No longer used in prophylaxis and chronic treatment of asthma in adults and children | Dyspepsia, increased liver enzymes | Contraindicated in patients with active liver disease or elevated liver enzymes | Recently withdrawn from the market |
| **LEUKOTRIENE RECEPTOR ANTAGONISTS**<br>*Mechanism—Block cysteinyl leukotriene receptor* | | | | |
| **Montelukast** | Prophylaxis and chronic treatment of asthma in adults and children | Hypersensitivity, headache, GI distress, pain | Monitor dosing when coadministered with P450 inducer | Montelukast and zafirlukast are not for acute asthma attacks and are generally not appropriate as monotherapy for asthma. Both drugs excreted in breast milk. |
| **Zafirlukast** | Prophylaxis and chronic treatment of asthma in adults and children | Hypersensitivity, headache, GI distress, pain | Monitor PT when coadministered with warfarin | |

# 41

# Histamine Pharmacology

Ashish Sahasrabudhe and Robert R. Rando

**Introduction**
**Case**
**Physiology of Histamine**
- Histamine Synthesis, Storage, and Release
- Actions of Histamine
- Histamine Receptors

**Pathophysiology**
- Clinical Manifestations of Histamine Pathophysiology
- Histamine and Anaphylaxis

**Pharmacologic Classes and Agents**
- Strategies of Histamine Pharmacology
- H1 Receptor Antagonists
- H2 Receptor Antagonists
- H3 Receptor Antagonists

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Histamine is a biogenic amine found in many tissues. It is an autacoid; that is, a molecule secreted locally to increase or decrease the functional activity of nearby cells. *Histamine is a major mediator of inflammatory processes; it also has a significant role in regulating gastric acid secretion and in neurotransmission.* Knowledge of the diverse and multiple actions of histamine has led to the development of a number of important pharmacologic agents that regulate the effects of histamine in pathologic states.

## Case

Ellen, a 16-year-old high school student, suffers from allergic rhinitis. Every spring, she develops a runny nose, itchy eyes, and sneezing. To relieve her symptoms, she takes an over-the-counter antihistamine, diphenhydramine. She is unhappy with the side effects that accompany her allergy medication. Every time she takes a pill, Ellen feels drowsy and has a dry mouth. She makes an appointment with her doctor who, after allergy testing, advises taking loratadine. After taking her new allergy medication, Ellen's symptoms are relieved and she experiences no drowsiness. Ellen continues to take loratadine with no adverse side effects.

### QUESTIONS

1. Why does Ellen develop seasonal rhinitis?
2. Why does diphenhydramine relieve Ellen's symptoms?
3. Why does diphenhydramine cause drowsiness?
4. Why doesn't loratadine cause drowsiness?

## PHYSIOLOGY OF HISTAMINE

### HISTAMINE SYNTHESIS, STORAGE, AND RELEASE

Histamine is synthesized from the amino acid L-histidine. The enzyme **histidine decarboxylase** catalyzes the decarboxylation of histidine to produce 2-(4-imidazolyl)ethylamine, commonly known as **histamine** (Fig. 41-1). The synthesis of histamine occurs primarily in mast cells and basophils of the immune system, enterochromaffin-like cells (ECL cells) in the gastric mucosa, and certain neurons that use histamine as a neurotransmitter. Once histamine is released, oxidative pathways in the liver rapidly degrade histamine into inert metabolites. A major metabolite of histamine, imidazoleacetic acid, can be measured in the urine and the level of this metabolite can be used to determine the amount of histamine that has been released systemically.

*Histamine synthesis and storage can be divided into two "pools:" a slowly turning over pool and a rapidly turning*

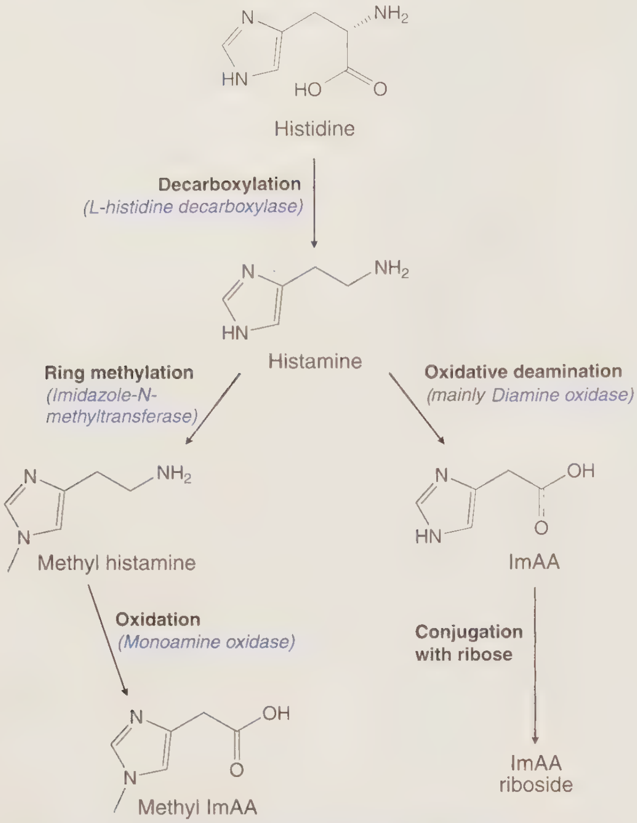


**Figure 41-1. Histamine Synthesis and Degradation.** Histamine is synthesized from histidine in a decarboxylation reaction catalyzed by L-histidine decarboxylase. The liver metabolizes histamine into inert byproducts. Histamine can be methylated on the imidazole ring or oxidatively deaminated. These degradation products can then undergo further oxidation or conjugation with ribose. Diamine oxidase is also known as histaminase. ImAA, imidazoleacetic acid.

*over pool.* The **slowly turning over pool** is located primarily in mast cells and basophils. In these inflammatory cells, histamine is stored in large granules, and the release of histamine involves complete degranulation of these cells. This process is termed the slowly turning over pool because several weeks are required to replenish the stores of histamine after degranulation has occurred. The **rapidly turning over pool** is located in gastric ECL cells and in histaminergic CNS neurons. These cells synthesize and release histamine as required for gastric acid secretion and neurotransmission, respectively. Unlike mast cells and basophils, ECL cells and histaminergic neurons do not store histamine. Instead, the production of histamine in these cells depends on physiologic stimuli. For example, in the gut, histidine decarboxylase is activated after the ingestion of food.

## ACTIONS OF HISTAMINE

Histamine has a wide spectrum of actions involving many organs and organ systems. To understand the role of histamine, it is useful to consider the physiologic effects of histamine in each individual tissue (Table 41-1). These effects can be organized into actions on smooth muscle, vascular endothelium, afferent nerve terminals, the heart, gastrointestinal tract, and CNS.

*The cellular actions of histamine on smooth muscle cause some muscle fibers to contract and others to relax.* Histamine causes contraction of **bronchial smooth muscle** in humans (although the effect varies in other species). The sensitivity of bronchial smooth muscle to histamine also varies among individuals; those with asthma may be up to 1,000-fold more sensitive to histamine-mediated bronchoconstriction than nonasthmatic individuals. Additional actions of histamine on smooth muscle involve both dilation and constriction of blood vessels. Histamine dilates all terminal arterioles and postcapillary venules. Veins, however, constrict on exposure to histamine. The dilatory effect on the postcapillary venule bed is the most prominent effect of histamine on the vasculature. During infection or injury, histamine-induced venule dilation engorges the local microvasculature with blood, enhancing the access of immune cells that initiate the repair processes in the damaged area. This engorgement explains the redness observed in inflamed tissues. Other smooth muscles contract on exposure to histamine. For example, histamine causes contraction of smooth muscle in the bowel, bladder, iris, and uterus. Although these effects can be demonstrated experimentally, they are not thought to play a large role physiologically or clinically.

**TABLE 41-1** Major Physiologic Actions of Histamine

| TISSUE | EFFECT OF HISTAMINE | CLINICAL MANIFESTATIONS | RECEPTOR SUBTYPE |
|---|---|---|---|
| Lungs | Bronchoconstriction | Asthma-like symptoms | H1 |
| Vascular smooth muscle | Post-capillary venule dilation<br>Terminal arteriole dilation<br>Venoconstriction | Rubor | H1 |
| Vascular endothelial cells | Contraction and separation of endothelial cells | Edema, wheal response | H1 |
| Nerves | Sensitization of afferent nerve terminals | Itchiness, pain | H1 |
| Heart | Minor increase in heart rate and contractility | Minor | H2 |
| Stomach | Increased gastric acid secretion | Peptic ulcer disease, heartburn | H2 |
| CNS | Neurotransmitter | Circadian rhythms, wakefulness | H3 |

Histamine also causes contraction of vascular endothelial cells. *Histamine-induced contraction of vascular endothelial cells causes these cells to separate from one another, allowing the escape of plasma proteins and fluid from postcapillary venules and, thereby, causing* ***edema.*** Thus, histamine is a key mediator of local responses at sites of injury.

Peripheral sensory nerve terminals also respond to histamine. The sensations of itch and pain result from a direct depolarizing action of histamine on afferent nerve terminals. This effect is responsible for the pain and itch experienced after an insect bite or sting, for example.

The combined actions of histamine on vascular smooth muscle, vascular endothelial cells, and nerve terminals are responsible for the "**wheal and flare**" response noted following histamine release in the skin. Endothelial cell contraction causes the edematous wheal response, while the redness and pain result from vasodilation and sensory nerve stimulation.

The cardiac effects of histamine consist of minor increases in the force and rate of contraction. Histamine enhances $Ca^{2+}$ influx into cardiac myocytes, leading to increased inotropy. The increase in heart rate is caused by an increase in the rate of phase 4 depolarization in sinoatrial node cells.

In 1920, it was discovered that histamine is a potent stimulator of acid secretion by parietal cells in the gastric mucosa. It was later established that *histamine is one of three molecules that regulate acid secretion in the stomach, the others being gastrin and acetylcholine.* The primary role of histamine in the gastric mucosa is to potentiate gastrin-induced acid secretion. Activation of histamine receptors in the stomach leads to an increase in intracellular $Ca^{2+}$ in parietal cells and results in increased HCl secretion by the gastric mucosa.

Histamine also functions as a neurotransmitter in the CNS. Both histidine decarboxylase and histamine receptors are expressed in the hypothalamus, and histaminergic CNS neurons have numerous diffuse projections throughout the brain and spinal cord. Although the functions of histamine in the CNS are not well understood, the molecule is believed to be important in the maintenance of wakefulness and to act as an appetite suppressant.

## HISTAMINE RECEPTORS

*The actions of histamine are mediated by histamine binding to one of three receptor subtypes—H1, H2, and H3.* All three subtypes are seven-transmembrane, G protein-coupled receptors. The receptor isoforms differ in their second messenger pathways and tissue distributions (Table 41-2).

The **H1 receptor** activates G protein-mediated hydrolysis of phosphatidylinositol, leading to increased inositol trisphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ triggers the release of $Ca^{2+}$ from intracellular stores, increasing cytosolic $Ca^{2+}$ concentration and activating downstream pathways. DAG activates protein kinase C, leading to phosphorylation of numerous cytosolic target proteins. In some tissues, such as bronchial smooth muscle, the increased cytosolic $Ca^{2+}$ increases the contraction of smooth muscle by $Ca^{2+}$/calmodulin-mediated phosphorylation of the myosin light chain. In other tissues, especially precapillary arteriolar sphincters and postcapillary venules, the increase in cytosolic $Ca^{2+}$ causes smooth muscle relaxation by inducing the synthesis of nitric oxide (see Chapter 20, Pharmacology of Vascular Tone).

H1 receptors are involved chiefly in inflammatory and allergic reactions. They are expressed primarily on vascular endothelial cells and smooth muscle cells. Stimulation of the H1 receptor results in: (1) edema; (2) bronchoconstriction; and (3) sensitization of primary afferent nerve terminals, depending on the tissue type. The H1 receptor is also expressed on the presynaptic membranes of histaminergic neurons in the tuberomamillary nucleus of the hypothalamus, where it acts as an autoreceptor to inhibit further release of histamine. These neurons may be involved in the control of circadian rhythms and wakefulness.

*The major function of the* ***H2 receptor*** *is to mediate gastric acid secretion in the stomach.* This receptor subtype is expressed on parietal cells in the gastric mucosa, where it acts synergistically with gastrin and acetylcholine to regulate acid secretion (see Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease). H2 receptors are also expressed on cardiac muscle cells, on some immune cells, and on certain presynaptic neuronal membranes. H2 receptors in parietal cells activate a G protein-dependent cyclic AMP cascade, leading to enhanced proton pump-mediated delivery of protons into the gastric fluid.

Currently, little is known about the actions of the **H3 receptor** subtype. H3 receptors are believed to cause feedback inhibition of certain effects of histamine. For example, H3 receptors are expressed on presynaptic nerve terminals in the CNS, where they limit the release of histamine from histaminergic neurons. H3 receptors have also been shown to limit histaminergic actions in gastric mucosa and bronchial smooth muscle. These effects may be explained by a decrease in $Ca^{2+}$ influx as a consequence of H3 receptor stimulation.

## PATHOPHYSIOLOGY

*Histamine is an essential mediator of the immune and inflammatory response.* Histamine plays a prominent role in the **IgE-mediated hypersensitivity reaction,** also known as the **allergic reaction.** In a localized allergic reaction, an allergen

**TABLE 41-2** Histamine Receptor Subtypes

| RECEPTOR SUBTYPE | POST-RECEPTOR MECHANISM | DISTRIBUTION |
|---|---|---|
| H1 | Increased $IP_3$, DAG, and intracellular $Ca^{2+}$ | Smooth muscle, vascular endothelium, brain (autoreceptor) |
| H2 | Increased cAMP | Gastric mucosa, cardiac muscle, mast cells, brain |
| H3 | Decreased $Ca^{2+}$ influx | CNS and some peripheral nerves |

(antigen) first penetrates an epithelial surface (e.g., skin, nasal mucosa). The allergen can also be delivered systemically, as in the case of an allergic response to penicillin. The presence of an allergen stimulates B lymphocytes, with the aid of T helper ($T_H$) cells, to produce IgE antibodies that are specific for that allergen. The IgE then binds to Fc receptors on mast cells and basophils, in a process known as **sensitization.** Once these immune cells are "sensitized" with IgE antibodies, they are able to detect and respond rapidly to a subsequent exposure to the allergen. Upon such a subsequent exposure, the allergen binds to and crosslinks the IgE/Fc receptor complexes, triggering cell degranulation (Fig. 41-2).

Histamine released by mast cells and basophils binds to H1 receptors on vascular smooth muscle cells and vascular endothelial cells. Activation of these receptors increases local blood flow and vascular permeability. This completes the initial stage of the inflammatory response: prolonged inflammation requires the activity of other immune cells as well. The local vasodilation allows such immune cells greater access to the injured area, while the increased vascular permeability facilitates entry of the immune cells into the tissue.

*Mast cell degranulation can also occur as a response to local tissue damage, in the absence of a humoral immune response.* For example, physical disruption of the mast cell membrane because of trauma or chemical damage can initiate the degranulation process. Histamine release allows for enhanced access of macrophages and other immune cells that can begin to repair the damaged area.

## CLINICAL MANIFESTATIONS OF HISTAMINE PATHOPHYSIOLOGY

The IgE-mediated hypersensitivity reaction is responsible for the initiation of certain inflammatory diseases, including **allergic rhinitis** and **acute urticaria** (hives). Recall that, in the introductory case, Ellen suffered from allergic rhinitis, with a runny nose, itchy eyes, and sneezing. In allergic rhinitis, an environmental allergen, such as pollen, crosses the nasal epithelium and enters the underlying tissue. There, the allergen encounters previously sensitized mast cells and crosslinks IgE/Fc receptor complexes on the mast cell surface. Consequently, the mast cell degranulates and releases histamine, which binds to H1 receptors in the nasal mucosa and local tissues. Stimulation of the H1 receptors causes blood vessel dilation and increases vascular permeability, leading to edema. This swelling in the nasal mucosa is responsible for the nasal congestion that is experienced in allergic rhinitis. The accom-

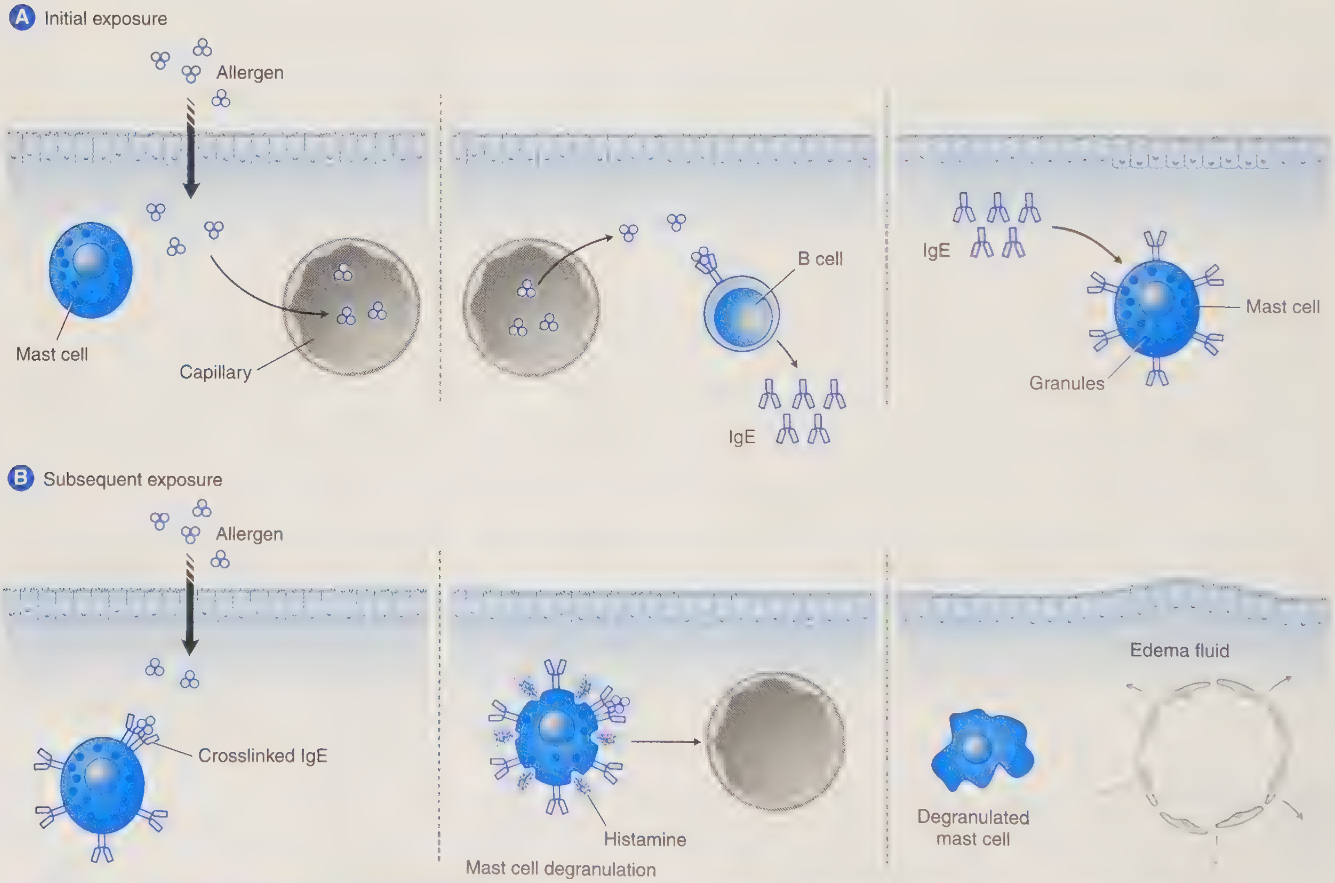


**Figure 41-2. Pathophysiology of the IgE-Mediated Hypersensitivity Reaction.** Allergen-induced mast cell degranulation requires two separate exposures to the allergen. **A.** On initial exposure, the allergen must penetrate mucosal surfaces so that it can encounter cells of the immune system. Activation of the immune response causes B lymphocytes to secrete allergen-specific IgE antibodies. These IgE molecules bind to Fc receptors on mast cells, leading to sensitization of the mast cells. **B.** On subsequent exposure, the multivalent allergen crosslinks two IgE/Fc receptor complexes on the mast cell surface. Receptor crosslinking causes the mast cell to degranulate. Local histamine release results in an inflammatory response, shown here as edema.

panying itching, sneezing, runny nose, and tearing result from the combined action of histamine and other inflammatory mediators, including kinins, prostaglandins, and leukotrienes. These molecules initiate the characteristic hypersecretion and irritation involved in allergic rhinitis.

A similar mechanism of mast cell activation occurs in acute urticaria. Here, an allergen, such as penicillin, enters the body, either through ingestion or parenterally, and reaches the skin through the circulation. Histamine release results in a disseminated wheal and flare response, creating red and itchy patches on the skin.

## HISTAMINE AND ANAPHYLAXIS

Systemic mast cell degranulation can cause the life-threatening condition known as **anaphylaxis.** Typically, anaphylactic shock is initiated by a hypersensitivity reaction to an insect bite, an antibiotic such as penicillin, or ingestion of certain highly allergenic foods (e.g., nuts). An allergen that is distributed systemically, either by intravenous injection or by absorption into the circulation, can stimulate mast cells and basophils to release histamine throughout the body. The resulting global vasodilation causes a massive decrease in blood pressure; hypotension also results from the systemic pooling of fluid because of the extravasation of plasma into the interstitium. Severe bronchoconstriction and epiglottal swelling are also induced by massive histamine release. This state of anaphylactic shock can be lethal within minutes if not treated rapidly by the administration of epinephrine (as described below).

# PHARMACOLOGIC CLASSES AND AGENTS

## STRATEGIES OF HISTAMINE PHARMACOLOGY

*Histamine pharmacology employs three approaches, each of which leads to inhibition of the actions of histamine* (Table 41-3). The first strategy is to administer a drug that functionally counteracts the effects of histamine. An example of this approach is the use of epinephrine to treat anaphylaxis. Epinephrine, an adrenergic agonist, induces bronchodilation and vasoconstriction (see Chapter 8, Adrenergic Pharmacology); these actions counter the brochoconstriction, vasodilation, and hypotension caused by histamine in anaphylactic shock. A second strategy is to prevent mast cell degranulation induced by binding of an antigen to the IgE/Fc receptor complex on mast cells. Cromolyn and nedocromil use this strategy to prevent asthma attacks (see Chapter 45, Integrative Inflammation Pharmacology: Asthma); these compounds disrupt the chloride current across mast cell membranes, which is a key step in the degranulation process.

Third, and the most frequently used approach, is to administer **histamine receptor antagonists,** which typically are selective for the H1, H2, or H3 receptor. These drugs are competitive antagonists of the histamine receptor: the antagonist competes with histamine for binding to the receptor and, thereby, inhibits histamine's agonist action. H1-selective antagonists are used in the treatment of allergic diseases, while H2-selective antagonists are used in the treatment of peptic ulcer disease (see Chapter 44).

## H1 RECEPTOR ANTAGONISTS

The finding that histamine is a major mediator of the allergic hypersensitivity reaction led to the discovery of the first **H1 receptor antagonist** by Bovet and Staub in 1937. Clinically useful drugs that inhibit the actions of histamine began to appear in the 1940s. It was recognized that none of these early drugs effectively inhibits histamine stimulation of gastric acid secretion. Anticipating that different receptors were involved in the latter effect, the term "H1 receptor antagonist" was proposed to replace the term "antihistamine." *Currently, H1 receptor antagonists parse into two categories: first generation and second generation H1 receptor antagonists.*

**First generation H1 antagonists** were the initial "antihistamines" to be used clinically. Their basic structure consists of two aromatic rings linked to a substituted ethylamine backbone. These drugs are divided into six main subgroups based on their substituted side chains (Fig. 41-3). First generation drugs are differentiated from second generation drugs by their side effect profiles. Because of their high lipophilicity, first generation antagonists readily penetrate the blood–brain barrier and exert inhibitory effects on H1 receptors in the central nervous system (especially the hypothalamus) as well as the periphery. The high CNS penetration accounts for the sedating effects of these drugs. In the introductory case, Ellen experienced sedating effects when she took **diphenhydramine** for her allergic rhinitis. The sedating effects of these drugs can be employed to treat insomnia: **doxylamine,** for example, is an H1 receptor antagonist that is available as an over-the-counter sleep aid. Other side effects, including drying of the nasal mucosa and xerostomia, are a result of weak anticholinergic effects. Many of the over-the-counter antihistamines are

**TABLE 41-3** Strategies of Histamine Pharmacology

| STRATEGY | EXAMPLE OF PHARMACOLOGIC AGENT | EXAMPLE OF DISEASE TREATED |
|---|---|---|
| Administer drug to counter the pathologic effect of histamine | Epinephrine | Anaphylaxis |
| Prevent mast cell degranulation | Cromolyn, nedocromil | Asthma |
| Histamine receptor antagonist | Diphenhydramine<br>Cimetidine, ranitidine | Allergies<br>Ulcers, heartburn |

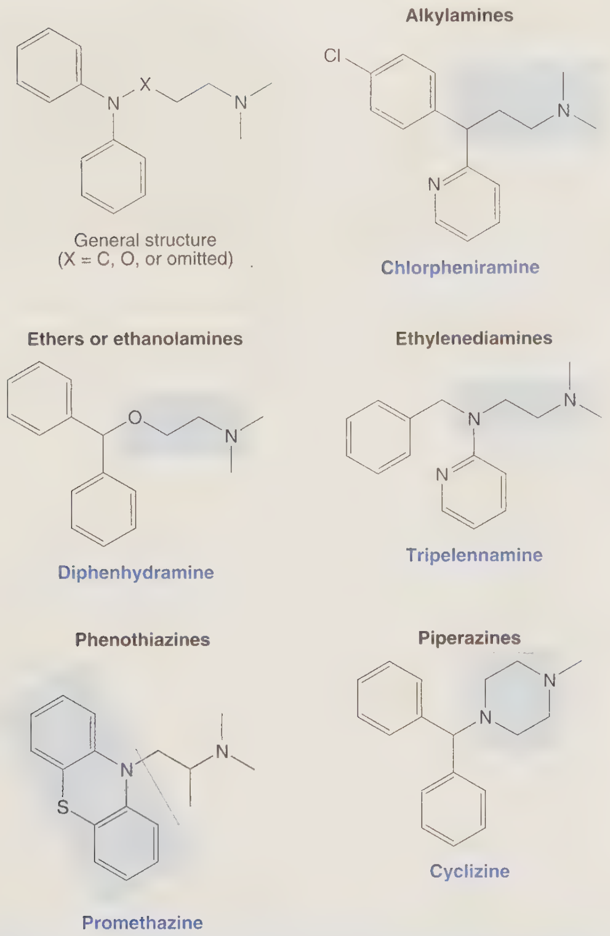


**Figure 41-3. Structure of First-Generation Histamine H1 Receptor Antagonists.** The general structure of the first-generation H1 receptor antagonists consists of a substituted ethylamine backbone with two terminal aromatic rings (Note the similarity between the ethylamine moiety in these antagonists and the ethylamine side chain of histamine shown in Fig. 41-1). Each of the five subclasses is a variation on this general structure. First-generation H1 antagonists are neutral compounds at physiologic pH and readily cross the blood–brain barrier. In contrast, second-generation H1 antagonists (e.g., loratadine, cetirizine, fexofenadine) are ionized at physiologic pH and do not appreciably cross the blood–brain barrier (not shown). This difference in blood–brain barrier penetration underlies the differential extent of sedation associated with use of the first- and second-generation H1 antagonists.

first generation H1 antagonists. Two of the more frequently used agents are diphenhydramine and **chlorpheniramine.**

**Second generation H1 antagonists** have decreased CNS penetration compared with first generation H1 antagonists. The low CNS penetration is attributable to two features of the molecules. First, these compounds are ionized at physiologic pH and, thus, do not diffuse well across membranes. Second, they exhibit high binding to albumin and, thus, are less free to diffuse into the CNS. Thus, these drugs do not possess the sedating side effect observed with first generation antagonists. **Loratadine** is the most well-known drug of this class.

There are, however, certain notable side effects and toxicities of the second generation H1 antagonists. Some of the earlier drugs of this class had serious cardiotoxic effects at high plasma concentrations. Two of these drugs, **terfenadine** and **astemiozole,** were withdrawn by the FDA because they caused prolonged QT intervals that sometimes led to ventricular arrhythmias. Second generation H1 antagonists are metabolized by hepatic cytochrome P450. Therefore, other drugs that are detoxified by the P450 system or that affect P450 activity should be used with caution in combination with second generation H1 antagonists.

Both first and second generation H1 antagonists are used to relieve the symptoms (edema, itching, pain) associated with allergic rhinitis, urticaria, and atopic allergy. Unfortunately, these drugs are ineffective in alleviating the bronchoconstriction associated with asthma. Other autacoids, including leukotriene B4, serotonin, and certain peptide mediators, are released in the lungs during an asthma attack, and the effects of these antacoids are not antagonized by H1 blockers.

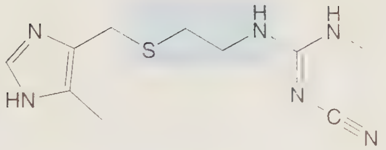
Some clinical situations favor the use of first generation versus second generation H1 receptor antagonists. First generation antagonists are often favored for acute use because they are inexpensive and available without a prescription and because they dry mucous membranes with their anticholinergic actions; the latter is a beneficial side effect when the drugs are taken for relief of a runny nose during allergic rhinitis. Second generation H1 receptor antagonists are often preferred for extended use because of their limited sedative effects.

## H2 RECEPTOR ANTAGONISTS

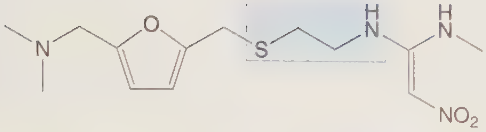
As noted above, H1 receptor antagonists do not affect gastric acid secretion. A great deal of excitement was generated by the development of drugs (burimamide, metiamide, and, later, **cimetidine**) that act as selective **H2 receptor antagonists** to inhibit histamine-induced gastric acid secretion. These molecules differ in structure from the H1 receptor antagonists, given that they contain an intact 5-membered (e.g., imidazole) ring and an uncharged side chain (Fig. 41-4). They act as competitive antagonists of histamine binding to H2 receptors on gastric parietal cells. This antagonism reduces acid secretion in heartburn and peptic ulcer disease. Many of these agents are now also available over-the-counter for the symptomatic treatment of heartburn. Cimetidine and **ranitidine** are two of the most commonly used H2 receptor antagonists, although **famotidine** and **nizatidine** are also prescribed. A significant side effect of cimetidine involves competition for cytochrome P450-mediated drug metabolism, which can result in undesirable elevations in the plasma levels of concomitantly administered drugs. H2 receptors are also expressed in the CNS and in cardiac muscle, but the therapeutic doses of H2 receptor antagonists are sufficiently low that cardiovascular and CNS side effects are negligible. Refer to Chapter 44 for a more detailed discussion of H2 receptor antagonists, their indications, and their toxicities.

## H3 RECEPTOR ANTAGONISTS

Recently, H3 receptors have been shown to mediate certain CNS and gastrointestinal effects. Pharmacologic agents selective for these receptors are in development, with potential applications in the treatment of obesity and attention-deficit disorders.



Cimetidine



Ranitidine

**Figure 41-4. Structure of Histamine H2 Receptor Antagonists.** H2 receptor antagonists have a thioethanolamine backbone (highlighted in blue box) that is N-substituted with a bulky side chain and terminates in a single five-membered ring. (Compare the bulky N-substituted side chain of the H2 antagonists with the simple tertiary amine of the H1 antagonists in Fig. 41-3, and compare the small five-membered imidazole or furan ring of the H2 antagonists with the pair of bulky aromatic rings of the H1 antagonists.) These structural differences enable cimetidine, ranitidine, and other H2 antagonists to bind selectively to H2 receptors in the gastric mucosa and, thereby, to decrease the production of gastric acid.

## *Conclusion and Future Directions*

The discovery of histamine and its receptors has significantly increased the pharmacologic options for treatment of allergy and peptic ulcer disease. Importantly, selective receptor targeting allows specific treatment of each of these disease processes without affecting the other physiologic actions of histamine. Drug selectivity is achieved by the existence and targeting of histamine receptor subtypes (H1, H2, H3).

The future of histamine pharmacology lies in the development of new H1 antagonists that do not possess cardiac side effects, but that do maintain the nonsedating effects of currently available agents. Future research will also focus on further elucidating the functional importance of H3 receptors.

## *Suggested Readings*

Kaliner M, Sloan R. American Family Physicians Monograph: Allergic Rhinitis. New York: Healthmark Medical Education Media, 1992. (*Discussion of the pathophysiology of allergic rhinitis.*)

Nicolas JM. The metabolic profile of second-generation antihistamine. Allergy 2000;55:46–52. (*Discussion of differences among second-generation drugs.*)

Timmerman H. Factors involved in the absence of sedative effects by the second generation antihistamines. Allergy 2000;55:5–10. (*Discussion of second-generation drugs.*)

**Drug Summary Table** **Histamine Pharmacology**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **FIRST GENERATION H1 RECEPTOR ANTAGONISTS** | | | | |
| *Mechanism—Competitively inhibit binding of histamine to H1 receptors* | | | | |
| Diphenhydramine | Allergy | Moderate somnolence, drying of nasal mucosa, xerostomia | All first generation H1 receptor antagonists are contraindicated for use when driving or operating heavy machinery | Common OTC medication for allergy |
| Chlorpheniramine | Allergy | Mild somnolence | | Common OTC medication for allergy |
| Doxylamine | Insomnia | Severe somnolence | | Common OTC medication for insomnia |
| **SECOND GENERATION H1 RECEPTOR ANTAGONISTS** | | | | |
| *Mechanism—Competitively inhibit binding of histamine to H1 receptors* | | | | |
| Loratadine | Allergy | | | Long duration |
| Fexofenadine | Allergy | | | Prescription required |
| Cetirizine | Allergy | | | Low risk of arrhythmia, prescription required |
| Terfenadine | No longer used in the treatment of allergy | Prolongation of QT interval, ventricular arrhythmia | Macrolide antibiotics, ketoconazole, fluconazole | Withdrawn by the FDA |
| Astemizole | Allergy | Prolongation of QT internal, ventricular arrhythmia | | Withdrawn by the FDA |
| **H2 RECEPTOR ANTAGONISTS** | | | | |
| *Mechanism—Competitively inhibit binding of histamine to H2 receptors* | | | | |
| Cimetidine<br>Ranitidine<br>Famotidine | Peptic ulcer disease, gastric ulcer, GERD, Zollinger–Ellison syndrome, heartburn | Diarrhea, dizziness, somnolence, CNS dysfunction, inhibition of hepatic cytochrome P450 | Drugs metabolized by P450, including warfarin, theophylline, beta-blockers, tricyclic antidepressants | Cimetidine has the most side effects and toxicities of all H2 receptor antagonists |

# 42

# Pharmacology of Hematopoietic and Immune Cell Stimulation

Ramy A. Arnaout and George D. Demetri

**Introduction**
**Case**
**Physiology of Hematopoiesis**
- Central Role of Growth Factors
  - *General (Multilineage) Growth Factors*
  - *Lineage-Specific Growth Factors*
- Red Blood Cell Production (Erythropoiesis)
  - *Erythropoietin*
- White Blood Cell Production (Myelopoiesis and Lymphopoiesis)
  - *Granulocyte-Stimulating Factors*
  - *Lymphocyte-Stimulating Factors*
- Platelet Production (Thrombopoiesis)
  - *Thrombopoietin*

**Pharmacologic Classes and Agents**
- Erythropoietic Agents
  - *Recombinant Human Erythropoetin (rhEPO) and Darbepoetin (NESP)*
- Fetal Hemoglobin (HbF)-Inducing Agents
  - *5-Azacytidine*
  - *Hydroxyurea*
  - *Butyrates*
- Antineutropenic (Myelopoietic) Agents
  - *Recombinant Human G-CSF (Filgrastim) and GM-CSF (Sargramostim)*
- Platelet-Inducing (Thrombopoietic) Agents
  - *Thrombopoietin Analogues*
  - *rhIL-11 (Oprelvekin)*
- Anticancer Agents
  - *Interferons*
  - *Levamisole*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

A number of clinical situations are marked by deficiencies of red blood cells, white blood cells, or platelets—the cells of the hematopoietic system. This chapter describes the pharmacologic agents that can be used to stimulate production of hematopoietic cells. (Nonpharmacologic alternatives include transfusion and bone marrow transplantation.) Blood cell production is controlled physiologically by hematopoietic growth factors, a diverse but functionally overlapping group of glycoproteins produced by the body in response to certain signals. For example, hypoxia stimulates production of the growth factor erythropoietin, which, in turn, stimulates the production of erythrocytes in an attempt to relieve the hypoxia. The main pharmacologic strategy used to stimulate the production of blood cells is to administer exogenous growth factors or synthetic growth factor analogues. This chapter provides a brief introduction to the cells of the hematopoietic system and the growth factors that stimulate their production and discusses the pharmacologic agents used to increase blood cell production. A brief summary of the possible application of interferons to cancer chemotherapy is also included.

## Case

Fifty-two-year-old Mrs. Morrow presents with lymph node-positive, localized early stage breast cancer, identified by pathology as an infiltrating ductal carcinoma. She begins adjuvant chemotherapy with doxorubicin and cyclophosphamide. Ten days after the first cycle of chemotherapy, her white blood cell count (WBC) drops, as expected; over the next 9 days, her WBC recovers to its normal value. By the third cycle of chemotherapy, Mrs. Morrow is moderately anemic, with a hematocrit of 28% (normal, 37–48%), and she feels quite fatigued. Seven days after the fourth cycle of chemotherapy, her WBC plummets to 800 cells per microliter of blood (normal, 4,300–10,800 cells/μL), and her absolute neutrophil count (ANC) is 300 cells/μL. In this setting, she develops shaking chills and a fever to 102°F. She is admitted to the hospital, where she receives parenteral antibiotics, and she remains there for 5 days until her ANC rises to an acceptable level. Mrs. Morrow completes her doxorubicin and cyclophosphamide chemotherapy, switches to paclitaxel, and receives local radiation therapy.

Mrs. Morrow is well for 2 years, but then presents with pain in the left leg. Workup reveals that the cancer has metastasized to her left femur and her liver. She is again fatigued, and her hematocrit is 27. She begins chemotherapy with doxorubicin and docetaxel, but again develops severe neutropenia and fever. Thereafter, her chemotherapy is supplemented with recombinant human G-CSF (filgrastim) and recombinant human erythropoietin (epoetin alfa). Her neutropenia and fever do not recur, and by 4 weeks after the initiation of erythropoietin therapy, her hematocrit has risen to 34.5, and she feels less fatigued. The chemotherapy yields excellent palliative results. Two years later, she is still in remission and leading an active life.

## QUESTIONS

- **1.** What are G-CSF and erythropoietin, and what are their mechanisms of action?
- **2.** How do recombinant growth factors differ from natural growth factors?
- **3.** What are some important side effects of recombinant growth factors?

## PHYSIOLOGY OF HEMATOPOIESIS

The cells of the hematopoietic system are functionally diverse (Table 42-1). Red blood cells, or **erythrocytes,** carry oxygen; many types of white blood cells, from **granulocytes** and **macrophages** to **lymphocytes,** fight infection and help protect against cancer; **platelets** help control bleeding. Nonetheless, these cells all have one feature in common: they all develop from a common cell in the bone marrow called the **pluripotent hematopoietic stem cell** (Fig. 42-1). Hematopoietic stem cells are induced to differentiate along committed lineages into red blood cells, white blood cells, or platelets through interactions with glycoproteins called **hematopoietic growth factors.**

## CENTRAL ROLE OF GROWTH FACTORS

Hematopoietic growth factors and cytokines comprise a heterogeneous group of molecules. Nearly three dozen growth factors have been identified, ranging in size from 9 to 90 kDa. The membrane-associated receptors for these growth factors

**TABLE 42-1** Hematopoietic Cells, Growth Factors, and Growth Factor Analogues

| CELL TYPE | MAJOR FUNCTION(S) | LINEAGE-SPECIFIC GROWTH FACTOR | DEFICIENCY STATE | THERAPEUTIC AGENT |
|---|---|---|---|---|
| Red blood cell (erythrocyte) | Oxygen transport | Erythropoietin (EPO) | Anemia | rhEPO, darbepoetin |
| Platelet (thrombocyte) | Hemostasis | Thrombopoietin (TPO) | Thrombocytopenia | rhTPO, IL-11, PEG-rHuMGDF (TPO analogue) |
| Monocyte/macrophage | Phagocytosis of bacteria and cellular and chemical debris, stimulation of T lymphocytes | M-CSF | —— | —— |
| Neutrophil | Phagocytosis of bacteria, immune stimulation | G-CSF | Neutropenia | Filgrastim, Sargramostim |
| Eosinophil | Control of parasites | IL-5 | —— | —— |
| Basophil | Phagocytosis of bacteria | | —— | Filgrastim, Sargramostim |
| B lymphocyte | Production of antibody, stimulation of T lymphocytes | Specific interleukins | Various immunodeficiency syndromes | —— |
| T lymphocyte | Killing of virus- and bacteria-infected cells, control of immune responses | Specific interleukins | Various immunodeficiency syndromes | rhIL-2 |
| NK cell | Killing of cancer cells | —— | —— | —— |

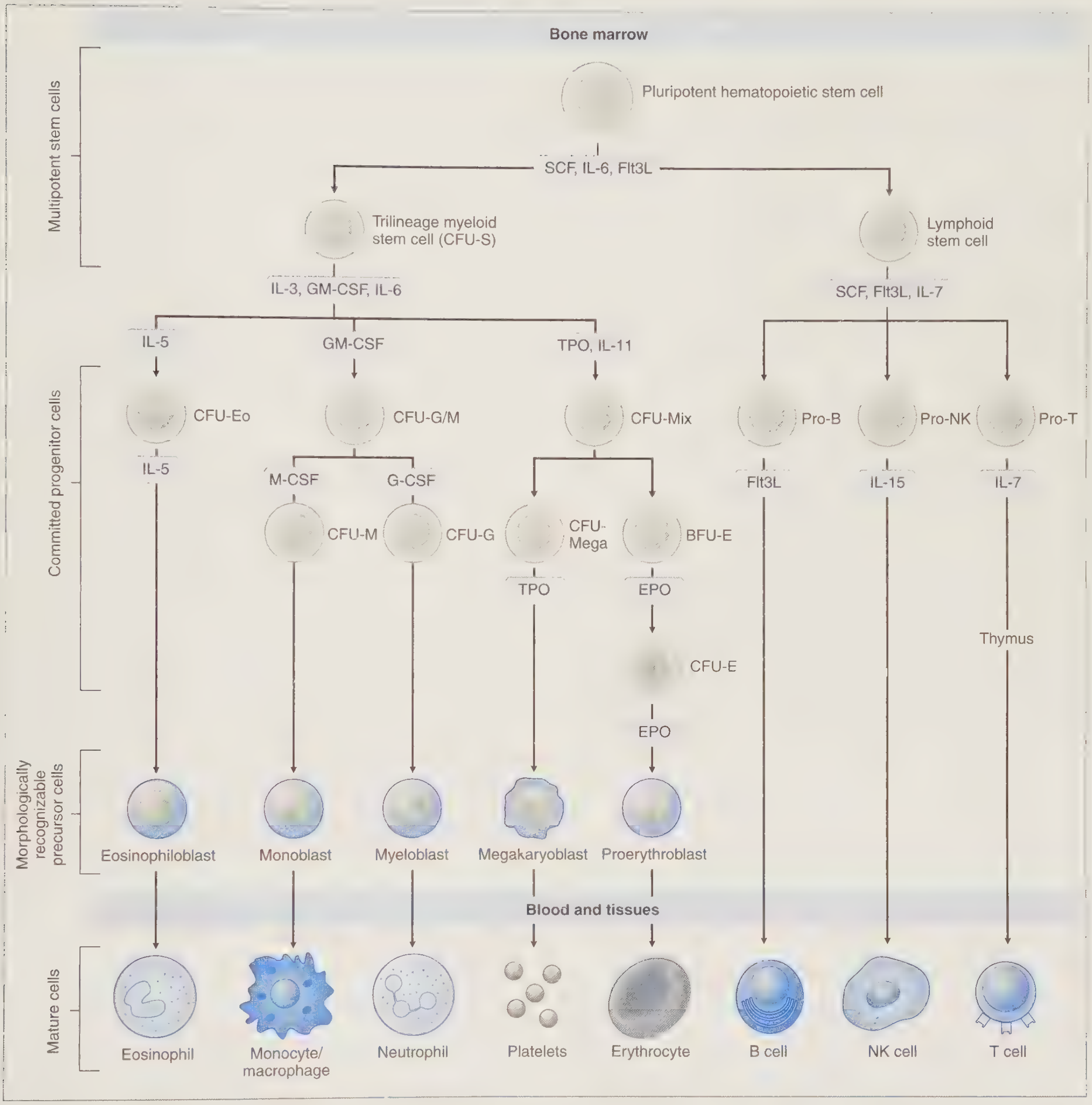

Bone marrow
Pluripotent hematopoietic stem cell
SCF, IL-6, Flt3L
Trilineage myeloid stem cell (CFU-S)
Lymphoid stem cell
IL-3, GM-CSF, IL-6
SCF, Flt3L, IL-7
IL-5
GM-CSF
TPO, IL-11
CFU-Eo
CFU-G/M
CFU-Mix
Pro-B
Pro-NK
Pro-T
IL-5
M-CSF
G-CSF
Flt3L
IL-15
IL-7
CFU-M
CFU-G
CFU-Mega
BFU-E
TPO
EPO
Thymus
CFU-E
EPO
Eosinophiloblast
Monoblast
Myeloblast
Megakaryoblast
Proerythroblast
Blood and tissues
Eosinophil
Monocyte/ macrophage
Neutrophil
Platelets
Erythrocyte
B cell
NK cell
T cell
Multipotent stem cells
Committed progenitor cells
Morphologically recognizable precursor cells
Mature cells

**Figure 42-1. Development of Cells of the Hematopoietic System.** Mature cells of the hematopoietic system all develop from pluripotent stem cells that reside in the bone marrow. The type of mature cell that develops is dependent on the extracellular milieu and the exposure of stem cells and progenitor cells to specific growth factors. The pluripotent stem cell differentiates into a trilineage myeloid stem cell (CFU-S) or a lymphoid stem cell. Depending on the growth factors that are present, CFU-S cells differentiate into granulocytes (eosinophils, monocyte/macrophages, neutrophils), platelets, or erythrocytes. Lymphoid stem cells differentiate into B cells, NK cells, or T cells. Except for the terminal differentiation of pro-T cells to mature T cells, which takes place in the thymus, the differentiation of all hematopoietic stem cells, progenitor cells, and precursor cells occurs in the bone marrow. Of the growth factors illustrated here, G-CSF, GM-CSF, erythropoietin (EPO), and IL-11 are currently used as therapeutic agents. BFU, burst forming unit. CFU, colony forming unit. CSF, colony stimulating factor. IL, interleukin. SCF, stem cell factor. TPO, thrombopoietin.

belong to six different receptor superfamilies, and genes encoding the growth factors are found on eleven different chromosomes. Functionally, hematopoietic growth factors stimulate the proliferation, differentiation, and function of hematopoietic cells. Certain factors selectively stimulate the growth and differentiation of a single lineage, as **erythropoietin** does for the erythrocyte lineage. Others, such as **stem cell factor,** stimulate the proliferation of multiple lineages. (Proteins with multiple effects are said to be **pleiotropic.**) Many growth factors act synergistically with one another, often with overlapping effects. This overlapping functionality may serve a protective role to ensure that vitally important processes, such as hematopoiesis, would be maintained if a gene for a single growth factor were to be altered by mutation.

Conceptually, growth factors can be divided into two groups: **general** (also called **multilineage** or **early-acting**) growth factors, which stimulate multiple lineages, and **lineage-specific** (also called **lineage-dominant** or **late-acting**) growth factors, which stimulate differentiation and survival of a single lineage.

## General (Multilineage) Growth Factors

General growth factors include **stem cell factor** (also called **steel factor** or **c-kit ligand**), **granulocyte-monocyte colony stimulating factor (GM-CSF), interleukin-3 (IL-3),** insulin-like growth factor 1, IL-9, IL-11, and others. Many of these growth factors are discussed below with respect to the development of individual hematopoietic cell types. The relevant pharmacologic principle is that general growth factors are appropriate for treating conditions in which multiple hematopoietic lineages are affected. (For example, in **pancytopenia,** there are inadequate numbers of cells in all lineages.)

The ability of general growth factors to stimulate multiple lineages results from two features of their molecular and cellular physiology. First, the receptors for these growth factors are both structurally related and modular; this commonality makes them somewhat interchangeable. Second, the signal transduction cascades stimulated by the binding of these growth factors to their receptors involve the same family of signaling proteins, the Jak-Stat protein kinases. Pharmacologists have exploited these commonalities to design synthetic growth factors with novel properties (see below).

## Lineage-Specific Growth Factors

For a growth factor to be lineage-specific, at least one of two conditions must be met: (1) the expression of the growth factor's receptor(s) must be limited to progenitor and/or precursor cells within a single lineage; or (2) the growth factor must induce inhibitory or apoptotic signals in cells of other lineages. Erythropoietin has already been introduced as one example of a lineage-specific growth factor; **thrombopoietin,** whose actions are essentially limited to the platelet lineage, is another. Other lineage-specific growth factors include **G-CSF,** which promotes the differentiation of neutrophils, and a number of **interleukins,** which have specific actions on certain myeloid and lymphoid lineages (see below). From a pharmacologic perspective, lineage-specific growth factors represent selective therapeutics that can be used to treat a deficiency of a single cell type. Some growth factors may also have unique effects against certain cancers; these effects seem to result from the prodifferentiation and promaturation properties of the growth factors.

# RED BLOOD CELL PRODUCTION (ERYTHROPOIESIS)

Erythrocytes are uniquely suited to their role of transporting oxygen from the lungs to the tissues of the body. These cells contain high concentrations of **hemoglobin,** a protein that binds and releases oxygen molecules in response to the partial pressure of oxygen in the blood. Each hemoglobin molecule consists of four similar polypeptide chains, and each chain contains a binding site for molecular oxygen. (The protein gets its name from the iron-containing **heme group** at each oxygen binding site, although heme groups are also found in many other proteins.) The major form of adult hemoglobin, which has two "alpha" and two "beta" chains ($\alpha_2\beta_2$), is called **hemoglobin A (HbA).** Fetal hemoglobin—or **hemoglobin F (HbF)**—contains gamma chains instead of beta chains ($\alpha_2\gamma_2$); this form of hemoglobin predominates during the last 6 months of fetal life. After birth, DNA methylation inactivates the γ globin gene, and expression of the β globin gene rises. It is important to note that the expression of the α, β, and γ globin chains is regulated independently, making possible a myriad of **hemoglobinopathies** in which the α or β chains are abnormal or underexpressed because of an inherited mutation. In **sickle cell anemia,** a point mutation in the β globin gene results in the production of an abnormal hemoglobin—**hemoglobin S (HbS)**—that polymerizes upon deoxygenation, causing morphologic "sickling" of red blood cells and leading to hemolytic anemia, painful (vaso-occlusive) crises, and profound end-organ damage. This autosomal recessive disease is the most common inherited blood disorder in the United States, affecting more than 70,000 individuals. Another common hemoglobinopathy is **β thalassemia,** in which the β chain is structurally and functionally normal, but underexpressed.

Upon their release from the bone marrow, normal erythrocytes circulate in the blood with a lifespan of approximately 120 days. The number of erythrocytes in the blood is determined by the balance between new erythrocyte production in the bone marrow and erythrocyte loss because of cell destruction (hemolysis) or bleeding. This number is measured clinically as either the hemoglobin level (the concentration of hemoglobin per unit volume of blood) or the **hematocrit** (the percentage of blood volume that is composed of erythrocytes). The normal hematocrit ranges from 42 to 50% in men and 37 to 48% in women; the gender difference is often attributed to physiologic—that is, menstrual—bleeding in women. A hemoglobin level or hematocrit below the normal range is defined as **anemia.**

## Erythropoietin

Erythrocyte production, or **erythropoiesis,** proceeds under the control of several growth factors. The major growth factor controlling erythropoiesis is erythropoietin, a heavily glycosylated protein that is produced mainly by the liver in the fetus and by the kidney after birth. A lineage-specific growth factor, erythropoietin has received great attention because it stimulates all but the earliest intermediates in the erythroid lineage but does not significantly affect other lineages. Its physiologic importance is attested to by experiments in mice and pathologic conditions in humans, which show that the absence of erythropoietin results in severe anemia.

Given the role of erythrocytes in transporting oxygen, it is not surprising that erythropoietin production is triggered by hypoxia. Exactly how cells in the peritubular capillary endothelium of the kidney sense hypoxia is still an open question, but the leading hypothesis is that there exists a specialized heme-containing protein that acts as an oxygen sensor (Fig. 42-2). In an incompletely understood reaction that involves several other proteins, this sensor protein is thought to activate transcription factors that upregulate erythropoietin synthesis and secretion. Among these transcription factors is

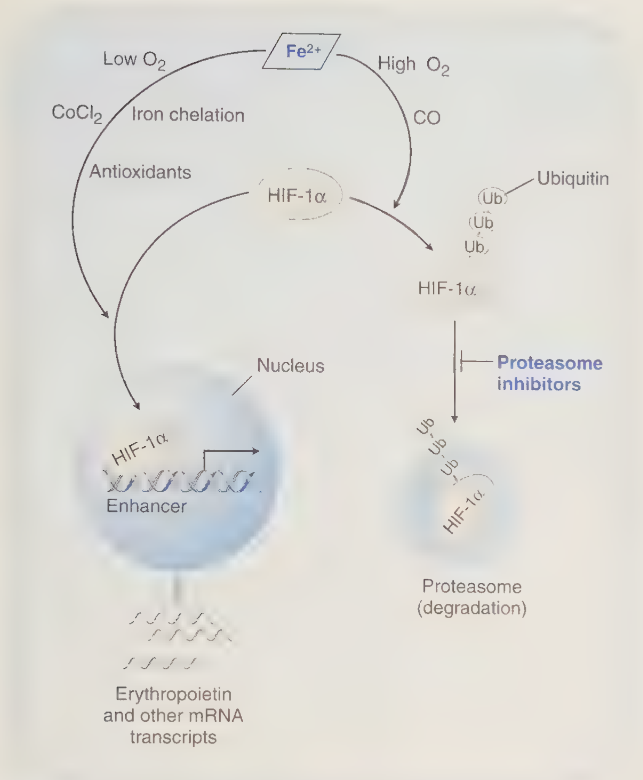


**Figure 42-2. Regulation of Erythropoietin Synthesis.** Synthesis of erythropoietin by the kidney is increased when the oxygen content of the blood is low and decreased when the oxygen content of the blood is high. The physiologic $O_2$ sensor has not been identified, but it is likely to be a heme-containing protein. (In vitro experiments using $CoCl_2$, iron chelation, antioxidants, and CO have been used to demonstrate the likely identity of the $O_2$ sensor as a heme-containing protein.) Low oxygen conditions cause this sensor to activate hypoxia-inducible factor 1α (HIF-1α), a transcription factor that translocates to the nucleus and increases the expression of a number of genes, including the gene encoding erythropoietin. Normoxic conditions result in ubiquitination (Ub) and proteolytic degradation of HIF-1α by the proteasome. In the absence of intranuclear HIF-1α, the erythropoietin gene is not transcribed, and erythropoietin synthesis is low. In pathologic conditions, such as chronic renal failure, the cells of the kidney that normally synthesize erythropoietin are damaged. These damaged cells cannot synthesize adequate amounts of erythropoietin, even under conditions of hypoxia, and anemia ensues. Recombinant human erythropoietin can be administered exogenously to supply the missing growth factor and, thereby, to treat the anemia.

hypoxia inducible factor 1 alpha (**HIF-1α**), which binds to an enhancer element of the erythropoietin gene. Following transcription and translation, the 166-amino acid, 18-kDa erythropoietin protein is glycosylated to 34-39 kDa, cleaved of its terminal arginine, secreted, and transported in the circulation to the bone marrow. There, it binds to erythropoietin receptors expressed on the surface of BFU-E and all subsequent progenitor and precursor cells in the erythroid lineage, including the erythrocyte's immediate precursor cell, the **reticulocyte.** Then, through a complex intracellular signaling cascade mediated by Jak-Stat, erythropoietin receptor activation enhances the terminal differentiation of reticulocytes to erythrocytes (Table 42-2).

Erythropoiesis completes a negative feedback loop on erythropoietin production, because the more erythrocytes in the blood—i.e., the higher the hemoglobin level and hematocrit—the higher the oxygen-carrying capacity of the blood. In the absence of cardiopulmonary disease, the higher oxygen-carrying capacity resolves the hypoxia and, thereby, removes the stimulus for increased erythropoietin production.

## WHITE BLOOD CELL PRODUCTION (MYELOPOIESIS AND LYMPHOPOIESIS)

White blood cells, or **leukocytes,** are the essential cells of the immune system. There are two main categories of leukocytes, corresponding to the two main branches of the immune system. Cells of the **innate branch** of the immune system include granulocytes (**neutrophils, eosinophils,** and **basophils**) **monocyte/macrophages,** and variants of the macrophage lineage. Neutrophils target bacteria, while eosinophils target parasites. Basophils participate in hypersensitivity responses. Macrophages also target bacteria, but these cells and their variants—**dendritic cells, Langerhans cells,** and **osteoclasts,** among others—have important additional functions. Macrophages have a key role in stimulating and regulating both branches of the immune system during infection and in clearing biological debris. Dendritic cells and Langerhans cells are important for initiating and targeting the immune response. These cells transport antigen from the **site of inoculation** to lymph nodes, where lymphocyte responses are coordinated. Osteoclasts are essential for bone resorption. Cells of the **adaptive branch** of the immune system are called **lymphocytes.** There are two types of lymphocytes: B cells, which make antibodies, and T cells, which target virus-infected and neoplastic cells (among other functions). "Adaptive" refers to the ability of these cells to recognize and respond to specific infectious agents and other targets (see Chapter 39, Principles of Inflammation and the Immune System).

All white blood cells develop from pluripotent hematopoietic stem cells (Fig. 42-1). Under the influence of growth factors, these stem cells differentiate into either **myeloid stem cells** or **lymphoid stem cells.** Myeloid stem cells further differentiate into the various cells of the innate branch of the immune system (as well as erythrocytes and platelets), while lymphoid stem cells differentiate into cells of the adaptive branch of the immune system. The growth factors that regulate these differentiation pathways are discussed below.

### Granulocyte-Stimulating Factors

The differentiation of pluripotent stem cells into myeloid stem cells is fostered by certain general growth factors such as stem cell factor and IL-3. Further differentiation of myeloid stem cells into neutrophils and monocyte/macrophages is controlled by lineage-specific growth factors, most notably **granulocyte colony-stimulating factor (G-CSF), granulocyte-monocyte colony-stimulating factor (GM-CSF),** and **monocyte-stimulating factor (M-CSF).** The differentiation of myeloid stem cells into eosinophils is controlled by **IL-5.**

G-CSF is an 18-kDa glycoprotein that, like other growth factors, signals through the Jak-Stat signaling cascade. G-CSF is released into the bloodstream by monocytes, macrophages, epithelial cells, and fibroblasts at sites of infection. In the bone

**TABLE 42-2** Physiologic Regulation of Erythropoiesis and Pathologic Conditions That Stimulate or Inhibit Erythropoiesis

| | PRECIPITATING CONDITIONS | |
|---|---|---|
| PHYSIOLOGIC REGULATION | STIMULATE ERYTHROPOIESIS | INHIBIT ERYTHROPOIESIS |
| 1. Hypoxia | Bleeding<br>Hemolysis<br>High altitude<br>Pulmonary disease | |
| 2. Erythropoietin synthesis in kidney | | Chronic renal failure |
| 3. Glycosylation, cleavage, and secretion of erythropoietin into circulation | | |
| 4. Stimulation of erythropoietin receptor on progenitor cells in bone marrow | | |
| 5. Intracellular Jak-Stat signaling cascade | | |
| 6. Erythroblast differentiation and erythrocyte production | | Iron, folate, or vitamin B12 deficiency<br>Anemia of chronic disease<br>Sideroblastic anemia<br>Thalassemia<br>Malignant infiltration of bone marrow<br>Aplastic anemia, pure red cell aplasia<br>Drug-induced bone marrow toxicity |
| 7. Resolution of hypoxia | | |

marrow, G-CSF stimulates the production of neutrophils, which in turn enhance the ability of the immune system to fight the infection. Locally released G-CSF stimulates neutrophil-mediated phagocytosis. These combined effects explain the benefit of G-CSF for Mrs. Morrow in the introductory case.

GM-CSF has broader effects than G-CSF. Produced mainly by macrophages and T cells, this 18–28 kDa glycoprotein stimulates the differentiation of myeloid stem cells and progenitor cells into morphologically recognizable precursors of eosinophils, monocyte/macrophages, and neutrophils. GM-CSF also enhances the activity of these mature leukocytes and promotes the differentiation of macrophages into Langerhans cells. Some of the effects of GM-CSF are indirect. For example, the effects of GM-CSF on neutrophil production and function may result not only from direct GM-CSF stimulation of neutrophil precursors, but also from the secretion of IL-1 by other GM-CSF-stimulated cells. Like G-CSF, GM-CSF signals through the Jak-Stat signaling pathway.

The effects of M-CSF are restricted to the differentiation and activation of monocyte/macrophages and their variants; in a positive feedback loop, these are also the cells that produce M-CSF. M-CSF exists in alternatively spliced 70–80 kDa and 40–50 kDa isoforms.

IL-5 is produced by a subset of helper T cells. This growth factor selectively promotes the differentiation, adhesion, degranulation, and survival of eosinophils. As such, IL-5 is believed to have an important role in the pathophysiology of allergic reactions and asthma.

## Lymphocyte-Stimulating Factors

Regulatory proteins called **interleukins** control lymphocyte development and activation. To date, more than fifteen members of this family have been defined. Family members are numbered IL-1, IL-2, and so forth. Interleukins regulate not only lymphocyte differentiation, but also multiple and overlapping aspects of the innate and adaptive immune responses, including stimulation of T cells and macrophages. Several interleukins are described above as granulocyte-stimulating factors; others are discussed below in the context of platelet production.

**IL-2** and **IL-7** are two interleukins that are critical to white blood cell differentiation. IL-2 is a 45-kDa protein produced by T cells. Because it drives proliferation of T cells and B cells, IL-2 had once received much attention as a potential immunostimulant. Investigations of this hypothesis showed, however, that mice lacking IL-2 exhibit lymphoproliferative rather than lymphopenic diseases. This unexpected finding underscores the principle that growth factors have diverse functions in vivo, including, in this case, regulatory or suppressive (tolerogenic) effects as well as stimulatory effects. This finding also points out that uncontrolled proliferation can ensue if differentiation is not regulated normally, a concept that may underlie some types of cancer. IL-7 is produced by cells in the spleen, thymus, and bone marrow stroma; it is a general lymphostimulatory growth factor that enhances the growth and differentiation of B cells and T cells.

A second family of regulatory proteins that modulate lymphocyte growth and activity is the **interferon** family. Like the interleukins, these proteins can stimulate the activity of T cells and macrophages. Interferons have prominent antiviral actions and are used in the treatment of such infections as hepatitis B and C. Other effects of interferons include promoting the terminal differentiation of lymphocytes, suppressing cell division (in some situations), and exerting direct cytotoxic effects on cells under stress. There are three types of interferons—called IFN-α, IFN-β, and IFN-γ–that have different biological actions. The cellular effects of interferons, like those of the growth factors, are mediated by specific cell surface receptors and Jak-Stat signal transduction cascades.

## PLATELET PRODUCTION (THROMBOPOIESIS)

Platelets—sometimes called **thrombocytes**—are essential for clot formation. These small cells have a half-life in the circulation of about 9 or 10 days. The production of platelets, like that of all formed elements of the hematopoietic system, is controlled by both general and lineage-specific growth factors (Fig. 42-3). The most important general growth factors that stimulate platelet production are IL-11, IL-3, GM-CSF, stem cell factor, and IL-6. Not surprisingly, these factors also stimulate the production of erythrocytes because platelets and erythrocytes share a common progenitor, the CFU-Mix cell. Whether CFU-Mix cells become erythrocytes or platelets depends on their subsequent exposure to lineage-specific growth factors. Differentiation into BFU-E and other cells of the erythroid lineage is promoted by erythropoietin. In contrast, differentiation into CFU-Mega cells and then into megakaryocytes (which then form platelets) is promoted by a specific growth factor, thrombopoietin (Fig. 42-1).

### Thrombopoietin

Thrombopoietin (TPO) is produced in the liver and, to a lesser extent, in the proximal convoluted tubule of the kidney. Like erythropoietin, thrombopoietin is a heavily glycosylated protein (35 kDa) that has its major effect on a single cell lineage; also like erythropoietin, thrombopoietin signals through a Jak-Stat transduction cascade. However, unlike erythropoietin, thrombopoietin is not regulated in its activity at the level of gene expression, because thrombopoietin is expressed constitutively. Instead, by an unusual mechanism, thrombopoietin levels are regulated by the thrombopoietin receptor, (also known as Mpl), which is the protein product of the gene *c-mpl.*

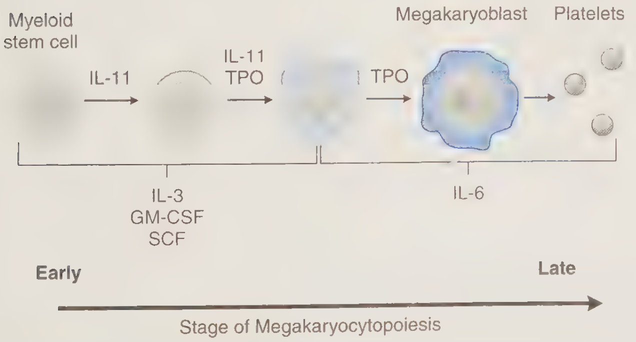


**Figure 42-3. Growth Factors Involved in Platelet Production.** A number of growth factors are involved in platelet production (megakaryocytopoiesis). IL-11 acts primarily in the early stages; this growth factor stimulates production of GM-CSF and acts synergistically with IL-3 and stem cell factor (SCF) to increase the proliferation and differentiation of megakaryocyte progenitors. IL-6 and thrombopoietin (TPO) act primarily in the late stages of megakaryocytopoiesis. Oprelvekin (recombinant human IL-11) can be used therapeutically to increase platelet production. Because IL-11 acts at an early stage in megakaryocytopoiesis, this drug requires a number of days to stimulate the production of new platelets. Recombinant TPO is also under development; this agent would be expected to increase platelet production within a shorter period of time.

Structurally and functionally, the thrombopoietin receptor resembles the receptors for IL-3, erythropoietin, and GM-CSF. It is found both on platelet progenitors—CFU-S, CFU-Mix, CFU-Mega, and megakaryocytes—and on platelets themselves. Thrombopoietin has different effects on these cell types, however. On platelet progenitors, the binding of thrombopoietin to the thrombopoietin receptor promotes cell growth and differentiation. On platelets, thrombopoietin receptors act as molecular sponges to bind excess thrombopoietin and, thereby, to prevent platelet overproduction if platelets are already in adequate supply. Thrombopoietin also enhances platelet function by sensitizing these cells to the proaggregatory effects of thrombin and collagen (see Chapter 21, Pharmacology of Hemostasis and Thrombosis).

## PHARMACOLOGIC CLASSES AND AGENTS

The hematopoietic growth factors used clinically can be divided into two groups. First, recombinant or synthetic growth factor analogues are used to treat deficiencies of the various hematopoietic cell populations. This group includes both the GM-CSF and erythropoietin received by Mrs. Morrow. Second, some growth factors have therapeutic use in the treatment of various malignancies.

## ERYTHROPOIETIC AGENTS

The erythroid-specific actions of erythropoietin make this growth factor an obvious candidate for use in the treatment of some forms of anemia. Anemia can result from any of a large number of underlying conditions that either interrupt the normal process of erythropoiesis or result in the premature loss or destruction of mature erythrocytes (Table 42-2). One common indication for erythropoietin therapy is chronic renal failure, in which the loss of functional kidney tissue results in loss of the cells responsible for erythropoietin production. Another potential indication for erythropoietin is cancer, which can induce a state of relative resistance to endogenous erythropoietin by mechanisms that may involve proinflammatory cytokines, oxidative stress, and anti-erythropoietin antibodies. (Cancer can also cause anemia through bleeding, poor nutrition, and infiltration of the bone marrow by tumor cells; these causes can often be diagnosed and treated directly.) Often, cancer-related anemia results from the toxicity of the chemotherapeutic agents used to treat the cancer. The fatigue associated with cancer-related anemia, such as that experienced by Mrs. Morrow, can therefore be treated with erythropoietin under some circumstances.

### Recombinant Human Erythropoietin (rhEPO) and Darbepoetin (NESP)

There are currently two erythropoietic agents, **recombinant human erythropoietin (rhEPO)** (also known as **epoetin alfa**) and **darbepoetin** (also known as novel erythropoiesis stimulating protein, or **NESP**), in clinical use. Like endogenous erythropoietin, both act by stimulating the erythropoietin receptor and inducing erythropoiesis. rhEPO increases the hematocrit level by ≥ 6%, in half to three-quarters of patients receiving the drug, depending on the etiology of the anemia and the dose of rhEPO administered. rhEPO and darbepoietin are similar in structure; in fact, the two agents differ only in the number of sialic acid (carbohydrate) groups that are attached to the protein. The development of darbepoietin began with the observation that sialic acid groups confer higher potency on erythropoietin. Darbepoietin's two extra sialic acid groups also give this drug a three-fold longer half-life than erythropoietin, enabling less frequent administration. Both agents are proteins and must, therefore, be administered parenterally.

## FETAL HEMOGLOBIN (HbF)-INDUCING AGENTS

Sickle cell disease is marked by acute "pain crises," increased susceptibility to infection, and profound hemolytic anemia. HbS-containing erythrocytes are the root cause of these clinical manifestations of the disease, which begins in childhood when HbS is first produced. Interestingly, newborns and infants are asymptomatic because fetal globin gene expression persists for many months, keeping HbF levels high (15% of total hemoglobin as late as 2 years of age). Consistent with this observation, adults in whom HbF expression persists at high levels have less frequent pain crises and milder anemia than those with low HbF expression. These observations have made increasing HbF levels a tantalizing therapeutic goal.

In principle, there are two approaches for increasing HbF: stimulating HbF expression in adults and preventing the switch from HbF to HbS in infants. Two drugs in current clinical practice, **5-azacytidine** and **hydroxyurea,** use the first approach; the **butyrates,** a class of drugs that are still in clinical trials, may utilize both approaches. Early studies suggest that 5-azacytidine and hydroxyurea could be synergistic with butyrates and with erythropoietin, although erythropoietin should be used with caution in patients with sickle cell disease because it stimulates erythropoiesis in HbS- as well as HbF-containing cells.

### 5-Azacytidine

5-Azacytidine is a DNA demethylating agent that, in the 1980s, was found to increase HbF production to over 20% of total globin expression in patients with sickle cell disease or β thalassemia. (HbF levels in adults with sickle cell disease are typically 1–5% of total hemoglobin; theoretical studies suggest that a level of 30–40% would render a patient asymptomatic.) 5-azacytidine is thought to act by reversing the methylation of the γ globin gene, but this mechanism remains unproven. Concern over the unknown mechanism of action and fear of long-term cancer risk (5-azacytidine also interferes with DNA synthesis; see Chapter 31, Pharmacology of Nucleotide Synthesis) have hindered the acceptance of this drug as a prophylactic therapy in sickle cell disease.

### Hydroxyurea

The 1990s saw the first use of hydroxyurea to treat sickle cell disease. A cytostatic agent that blocks cell division by inhibiting ribonucleotide reductase, hydroxyurea had previously been used to treat clonal hematologic disorders such as chronic myelogenous leukemia and polycythemia vera (see Chapter 31). From this experience, hydroxyurea was known to be relatively safe for long-term administration, even in children; myelosuppression of white blood cells and platelets was known to be the main side effect. The induction of HbF by hydroxyurea is slower than that by azacytidine; nevertheless, hydroxyurea has proved to be effective in about 60% of patients with sickle cell disease. In these patients, hydroxyurea increases HbF levels to greater than 20%, decreases the frequency of painful crises by 50% (from 4.5 to 2.5 per year, on average), and decreases the number of transfusions required by patients who have three or more crises per year. However, hydroxyurea does not prevent end-organ damage or stroke. In 1998, hydroxurea received FDA approval for use in the treatment of sickle cell disease.

Despite its long history, hydroxyurea's mechanism of action in sickle cell disease remains unknown. The current theory is that it blocks the division of HbS-expressing erythroid precursors, and that this somehow triggers reversion to a fetal pattern of hemoglobin expression in an attempt to maintain erythrocyte production. Interestingly, the mechanism by which hydroxurea increases HbF expression has been shown to be independent of ribonucleotide reductase inhibition.

### Butyrates

Butyrates (e.g., arginine butyrate, phenylbutyrate) are short-chain fatty acids that inhibit histone deacetylases, the enzymes that normally modify DNA to make it inaccessible to transcription factors. Butyrates have been shown to increase HbF levels from 2% to more than 20% in early clinical trials, although these agents are apparently not effective in patients whose baseline HbF level is less than 1%. In animal studies, butyrates have also been shown to prevent the switch from HbF to HbS. In a related observation, it has been found that children born to diabetic mothers (whose blood contains elevated levels of butyrates) have higher than normal levels of HbF. Butyrates are thought to act by allowing transcription factors to maintain or resume activity. Although this mechanism could explain the increased HbF production in response to butyrates, it does not explain the selectivity of butyrates for HbF production over HbS expression in patients with sickle cell disease.

## ANTINEUTROPENIC (MYELOPOIETIC) AGENTS

A low neutrophil count, or **neutropenia,** is most often the result of interference with progenitor cell division (**myelosuppression**). Neutropenia frequently accompanies leukemia and other malignancies that invade the bone marrow and it is a common side effect of cancer chemotherapy. Less common causes of neutropenia include bone marrow transplantation, congenital neutropenia, and HIV- or azidothymidine-associated neutropenia. Two agents have been approved for use in the treatment of cancer- and chemotherapy-induced neutropenia: recombinant human G-CSF (**filgrastim**), and recombinant human GM-CSF (**sargramostim**).

### Recombinant Human G-CSF (Filgrastim) and GM-CSF (Sargramostim)

Filgrastim and sargramostim are almost identical to the natural growth factors G-CSF and GM-CSF, and they act by the same mechanisms as the endogenous proteins. Although GM-CSF is a multilineage growth factor, the major clinical effect of GM-CSF or G-CSF administration is a dose-independent increase in the absolute neutrophil count. (GM-CSF also causes a mild and dose-dependent increase in eosinophils.) As noted above, G-CSF and GM-CSF enhance the microbicidal activity of neutrophils in addition to stimulating their production. The main side effect of G-CSF is bone pain, which resolves upon discontinuation of the drug; GM-CSF is associated with fever, arthralgia, edema, and pleural and pericardial effusion. Both drugs are proteins and must be administered parenterally; these drugs are typically administered daily over the course of several weeks. A recently introduced filgrastim analogue is conjugated to polyethylene glycol (PEG). This analogue, PEG-filgrastim, is metabolized more slowly than the native molecule. PEG-filgrastim can therefore be administered as a single injection that is functionally equivalent to multiple daily doses of filgrastim.

## PLATELET-INDUCING (THROMBOPOIETIC) AGENTS

A low platelet count, or **thrombocytopenia,** is an important dose-limiting side effect of many cancer chemotherapeutic agents. The complications of thrombocytopenia include increased bleeding risk and platelet transfusion requirement; platelet transfusion, in turn, is associated with an increased risk of infection, febrile reaction and, rarely, graft-versus-host disease.

Research into the pharmacologic management of chemotherapy-induced thrombocytopenia has focused on the thrombopoietin analogues **recombinant human thrombopoietin (rhTPO)** and **pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF),** although, to date, only **recombinant human IL-11 (rhIL-11 or oprelvekin)** has been approved for clinical use. These agents all have the potential to increase megakaryocytopoiesis (platelet production) in a dose-dependent fashion; although these drugs stimulate some multipotent as well as committed precursor cells, they do not significantly increase the hematocrit or white blood cell count. Importantly, these agents must all be administered prophylactically because their activity is delayed in onset, with a 1- to 3-week delay before the platelet count reaches its peak value. Current research has led to the identification of several new small molecule ligands for the TPO receptor, such as hydrazinonaphthalene and azonaphthalene, which could serve as lead compounds for the development of clinically useful drugs.

### Thrombopoietin Analogues

Cloning of the thrombopoietin gene in 1994 led to the development of two thrombopoietin analogues. The first, rhTPO, is a full-length, glycosylated analogue; the second, PEG-rHuMGDF, consists of the N-terminal 163 amino acids of thrombopoietin conjugated to polyethylene glycol (PEG). Like natural thrombopoietin, both rhTPO and PEG-rHuMGDF bind to Mpl (the endogenous receptor for thrombopoietin, named for its role in murine myeloproliferative leukemia); activation of Mpl is the basis for the effect of these drugs. Both rhTPO and PEG-rHuMGDF have been tested as prophylactic agents to minimize chemotherapy-induced thrombocytopenia, and both can cause a 2- to 10-fold increase in the platelet count. One caution is that stimulation of platelet production could lead to thrombosis as a potential side effect, if the platelets that are produced are also activated. A small trial of PEG-rHuMGDF suggests that this drug is safe to use in treating the thrombocytopenia associated with acute myelogenous leukemia (AML), although AML cells may also express the TPO receptor. The heavily bioengineered variants of natural TPO (e.g., PEG-rHuMGDF) have recently been dropped from clinical development because of an excess risk of developing anti-TPO autoantibodies, which could suppress natural platelet production. The testing of full-length rhTPO continues; there are no reports to date of neutralizing antibodies developing in patients who receive this lightly bioengineered agent, which differs from native human TPO only in its glycosylation pattern.

### rhIL-11 (Oprelvekin)

Although IL-11's ability to stimulate the differentiation of myeloid stem cells could theoretically make this protein a general growth factor, in clinical practice, recombinant human IL-11 (rhIL-11)—also called **oprelvekin**—has been used to stimulate platelet production. The recombinant form of IL-11, which is produced in *E. coli,* differs from natural IL-11 in that it lacks the N-terminal proline residue. rhIL-11 causes a dose-dependent increase in the platelet count and in the number of megakaryocytes in the bone marrow. The main clinical application of rhIL-11 is to prevent thrombocytopenia in patients who are about to receive chemotherapy. The practical goal of treatment is to maintain the platelet count above 20,000 per microliter (normal range, 150,000–450,000/μL) to minimize the risk of life-threatening bleeding. rhIL-11 use is associated with significant side effects, including fatigue and fluid retention. Atrial fibrillation has also been observed, and rhIL-11 should be used cautiously in any patient with underlying heart disease. The side effects of rhIL-11 likely result from pleiotropic effects of this factor on receptors distributed outside the hematopoietic system. It is unclear whether the therapeutic benefit of this agent outweighs the risk of unacceptable systemic side effects.

## ANTICANCER AGENTS

### Interferons

Clinical investigation has led to the use of interferons as therapeutic agents against a number of different malignancies (see Drug Summary Table), with moderate success. However, the multiple and overlapping effects of these proteins have made it difficult to determine the drugs' mechanism of action in any given clinical situation. Induction of antitumor-directed immunity, terminal differentiation of cycling tumor cells, and direct cytotoxic effects have all been postulated to have important roles in the treatment of different malignancies.

## Levamisole

**Levamisole** was known as an antihelminthic agent for decades before its anticancer effects were discovered. In combination with the antimetabolite 5-fluorouracil (see Chapter 31), this drug is now approved for use in the treatment of colon cancer. Although its mechanism of action is still unknown, levamisole is thought to cause macrophages and T cells to secrete cytokines (such as IL-1) and other factors that suppress tumor growth.

## *Conclusion and Future Directions*

The production of cells of the hematopoietic system—red blood cells (erythrocytes), white blood cells (neutrophils, monocytes, lymphocytes, and other cell types), and platelets—is controlled by a variety of proteins called growth factors. Cancer chemotherapy, malignant infiltration of the bone marrow, and other conditions can cause deficiencies (anemia, neutropenia, and/or thrombocytopenia) in these cell populations. The agents currently used to treat these deficiencies are recombinant analogues of the natural growth factors. Thus, the erythropoietin analogues rhEPO and darbepoetin treat anemia; rhIL-11 and the thrombopoietin analogue rhTPO treat thrombocytopenia; and the G-CSF and GM-CSF analogues filgrastim and sargramostim treat neutropenia. Several agents affecting the hematopoietic system are also used to treat sickle cell anemia, a common autosomal recessive disease caused by a point mutation in the β globin gene. These agents (hydroxyurea, 5-azacytidine) increase expression of the fetal isoform of β globin, raising levels of HbF and restoring erythrocyte structure and function. Several other drugs, including levamisole and recombinant forms of human immunostimulatory proteins called interferons, are used to treat certain cancers, although their precise mechanisms of action remain unknown. Teasing apart the complex overlapping functionalities of these proteins is likely to prove a source of more selective pharmacologic interventions in the future.

## *Suggested Readings*

Cwirla SE, Balasubramanian P, Duffin DJ, et al. Peptide agonist of the thrombopoietin receptor as potent as the natural cytokine. Science 1997;276:1696–1699. (*Discovery of two short peptides that have thrombopoietic activity in cell culture.*)

Demetri GD. Pharmacologic treatment options in patients with thrombocytopenia. Semin Hematol 2000;37:11–18. (*Reviews the therapy for thrombocytopenia.*)

Demetri GD. Anaemia and its functional consequences in cancer patients: current challenges in management and prospects for improving therapy. Br J Cancer 2001;84:31–37. (*Reviews the use and effectiveness of recombinant human erythropoetin.*)

Egrie JC, Browne JJ. Development and characterization of novel erythropoiesis stimulating protein (NESP). Br J Cancer 2001;84: 3–10. (*Reviews the development of darbepoetin.*)

Grander D, Einhorn S. Interferon and malignant disease—how does it work and why doesn't it always? Acta Oncol 1998;37: 331–338. (*Possible mechanisms of action of the interferons.*)

Lifton R, Bennett JM. Clinical use of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in neutropenia associated with malignancy. Hematol Oncol Clin North Am 1996;10:825–839. (*Clinical use of GM-CSF and G-CSF.*)

Ozer H, Armitage JO, Bennett CL, et al. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. J Clin Oncol 2000;18:3558–3585. (*2000 report of the American Society of Clinical Oncology Growth Factors Expert Panel.*)

Vansteenkiste J, Pirker R, Massuti B, et al. Double-blind, placebo-controlled, randomized phase III trial of darbepoetin alfa in lung cancer patients receiving chemotherapy. J Natl Cancer Inst 2002;94:1211–1220. (*Evidence for clinical effectiveness of darbepoetin.*)

Wingard JR, Demetri GD. Clinical Applications of Cytokines and Growth Factors. Norwell, MA: Kluwer, 1999. (*The biology and clinical use of growth factors.*)

**Drug Summary Table** Pharmacology of Hematopoietic and Immune Cell Stimulation

| Drug | Clinical Use | Side Effects/Toxicity | Interactions | Contraindications | Notes |
|---|---|---|---|---|---|
| **ERYTHROPOIETIC AGENTS**<br>*Mechanism—Stimulate erythroid differentiation and erythrocyte production by binding EPO receptor* | | | | | |
| Erythropoietin (rhEPO) | Anemia of chronic renal failure, cancer, or chemotherapy; preparation for autologous transfusion/transplant | | | | Highly glycosylated<br>Sialic acid on three residues |
| Darbepoetin | | | | | Highly glycosylated<br>Sialic acid on five residues |
| **HbF-INDUCING AGENTS**<br>*Mechanism—Unknown* | | | | | |
| Hydroxyurea | Sickle cell anemia and some cancers | Nausea and vomiting | | | Thought to block division of HbS precursors, triggering reversion to a HbF expression pattern<br>Thought not to induce HbF expression by inhibition of ribonucleotide reductase |
| **ANTINEUTROPENIC AGENTS**<br>*Mechanism—Stimulate neutrophil and macrophage production by binding G-CSF or GM-CSF receptor* | | | | | |
| Filgrastim (rhG-CSF) | Malignancy- and chemotherapy-induced neutropenia | Bone pain (generally well tolerated) | | | |
| Sargramostim (rhGM-CSF) | Malignancy- and chemotherapy-induced neutropenia | Fever, arthralgia, edema, pleural and pericardial effusion | | | Also causes dose-dependent eosinophilia |

*(Continued)*

**Drug Summary Table** **Pharmacology of Hematopoietic and Immune Cell Stimulation** *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **PLATELET-INDUCING AGENTS**<br>*Mechanism—Stimulate platelet production by binding TPO or IL-11 receptor* | | | | |
| rhTPO | Chemotherapy-induced thrombocytopenia | | | Full-length recombinant TPO analogue activates TPO receptor (Mpl) |
| rhIL-11 (oprelvekin) | Chemotherapy-induced thrombocytopenia | Fatigue, fluid retention, atrial fibrillation | | Stimulates platelet production by binding IL-11 receptor (gp130) |
| **ANTICANCER AGENTS**<br>*Mechanism—Unknown* | | | | |
| IFN-α | Hairy cell leukemia, malignant melanoma, carcinoid tumors, chronic myelogenous leukemia, Kaposi sarcoma, hepatitis B and C | Depressive illness, suicidal behavior, fatigue, flu-like symptoms, headache, nausea/vomiting, fever, chills | | |
| IFN-β | Relapsing type multiple sclerosis | Flu-like symptoms, fever, chills, anemia | | |
| IFN-γ | Chronic granulomatous disease | Fever, chills, headache, rash, nausea/vomiting | | |
| TNF-α | Malignant melanoma, soft tissue sarcoma of the extremities | | | |
| Levamisole | Colon cancer (usually with 5-fluorouracil) | | | Also used as an antiparasitic agent (see Chapter 37) |

# 43

# Pharmacology of Immunosuppression

Harris S. Rose, Ehrin J. Armstrong, Lloyd B. Klickstein, and Joren C. Madsen

- **Introduction**
- **Case**
- **Pathophysiology**
  - Transplantation
    - *Solid Organ Rejection*
      - *Hyperacute Rejection*
      - *Acute Rejection*
      - *Chronic Rejection*
    - *Graft Versus Host Disease*
  - Autoimmunity
- **Pharmacologic Classes and Agents**
  - Inhibitors of Gene Expression
    - *Glucocorticoids*
  - Cytotoxic Agents
    - *Antimetabolites*
      - *Azathioprine*
      - *Methotrexate*
      - *Mycophenolic Acid and Mycophenolate Mofetil*
      - *Leflunomide*
    - *Alkylating Agents*
      - *Cyclophosphamide*
  - Specific Lymphocyte Signaling Inhibitors
    - *Cyclosporine and Tacrolimus*
    - *Sirolimus*
  - Cytokine Inhibition
    - *TNF-α Inhibitors*
    - *IL-1 Inhibitors*
  - Antibodies
    - *Polyclonal Antibodies*
      - *Anti-Thymocyte Globulin*
    - *Monoclonal Antibodies*
      - *OKT3*
      - *Anti-CD25 mAb*
      - *Anti-CD52 mAb*
  - Inhibition of Costimulation
  - Blockade of Cell Adhesion
- **Conclusion and Future Directions**
- **Suggested Readings**

## INTRODUCTION

Patients with autoimmune disease, and patients who have received transplanted tissues or organs, typically require therapy with immunosuppressive drugs. Immunosuppressive agents have been in use for 50 years, beginning with corticosteroids, antimetabolites, and alkylating agents. These early agents assisted in the treatment of previously incurable conditions, but their lack of specificity led to many unwanted side effects. Over the past 20 years, the field of immunosuppression has shifted to specific inhibitors of immunity that affect distinct immune pathways. This shift is important both because of the greater efficacy and reduced toxicity of these agents, and because, as the mechanisms of these agents are discovered, insights are gained into the operation of the immune system.

This chapter is divided into two main sections. The pathophysiology section describes the mechanisms of disease that require immunosuppression. The pharmacology section describes the agents approved for use in the United States.

## Case

Mrs. White is 59 years old when she undergoes heart transplantation in the spring of 1990 for heart failure resulting from chronic severe mitral valve regurgitation. Her initial immunosuppressant regimen consists of cyclosporine, glucocorticoids, and azathioprine. Progress during the first 3 months post-transplant is excellent, but then Mrs. White develops anorexia, and an echocardiogram shows a significant drop in her cardiac ejection fraction. The glucocorticoid dose is increased, her ejection fraction improves, and she is discharged from the hospital.

Four months after surgery, Mrs. White is admitted with dyspnea and fatigue. A heart biopsy demonstrates evidence of moderate acute rejection, with localized areas of lymphocytic infiltration and necrosis. She is treated with a 10-day course of OKT3 (a monoclonal antibody against T cells) that produces side effects of fever, myalgias, nausea, and diarrhea. The patient also states, "This OKT3 makes me sleepy." Mrs. White is discharged following improvement of her cardiac status. A few months later, however, she returns to the hospital with dyspnea and fatigue. A biopsy shows no evidence of rejection, but based on her history and symptoms, rejection is suspected. She is tested for the presence of anti-OKT3 antibodies, but because no neutralizing antibodies are found, a second course of OKT3 is administered, and her symptoms abate.

In December 2000, Mrs. White arrives at the hospital for her regular annual examination. She is in good health, being maintained on a baseline immunosuppressant regimen of cyclosporine, azathioprine, and glucocorticoids, without evidence of rejection since 1990. Coronary angiography shows remarkably normal coronary arteries, perhaps the result of aggressive plasma lipid level maintenance by her physicians. However, Mrs. White's BUN and creatinine levels are elevated, an indication of chronic renal insufficiency. Her physicians decide to continue to monitor her kidney function closely, and she is discharged from the hospital.

## QUESTIONS

1. How does each of the drugs prescribed for Mrs. White reduce the likelihood of rejection?
2. Why does Mrs. White develop fever, myalgias, nausea, and diarrhea following administration of OKT3?
3. Before receiving her second course of OKT3, why is Mrs. White tested for neutralizing antibodies?
4. What is a likely cause of Mrs. White's chronic renal insufficiency?

# PATHOPHYSIOLOGY

## TRANSPLANTATION

The first transplant performed successfully in humans was a kidney transplant between identical twins. No immunosuppression was used, and the individuals did well. Currently, most organ transplantation occurs between unrelated individuals. Transplant rejection, although detrimental, is caused by the appropriate reaction of the immune system to an organ from a different individual of the same species. This is termed **alloimmunity,** and occurs when the recipient's immune system attacks a transplanted organ, or when donated bone marrow lymphocytes mount an assault on the recipient, as in graft versus host disease (GVHD).

### Solid Organ Rejection

Transplant rejection of solid organs can be divided into three major phases according to the time to onset. These phases, **hyperacute, acute, and chronic rejection,** are each caused by different mechanisms and are, therefore, treated differently. The following three sections examine each of these processes and Table 43-1 summarizes their differences.

#### *Hyperacute Rejection*

Preformed recipient antibodies to donor antigen mediate **hyperacute rejection.** Because these antibodies are present at the time of organ implantation, hyperacute rejection occurs almost immediately following reperfusion of the transplanted organ. In fact, the surgeon can observe the changes in the organ minutes after restoration of blood flow. The normal, healthy, pink appearance of the transplanted organ rapidly becomes cyanotic, mottled, and flaccid. The rapid change is the result of complement activation by antibody attachment to the endothelial cells of the transplanted organ, resulting in thrombosis and ischemia. The most common cause of hyperacute rejection is antibody reaction with blood type antigens of donor organs in recipients who do not express those antigens (i.e., type AB donor to a type O recipient). Matching blood types between donor and recipient prevents hyperacute rejection and, therefore, drug therapy for hyperacute rejection is typically not necessary.

#### *Acute Rejection*

Acute rejection has cellular and humoral components. **Acute cellular rejection** is mediated by cytotoxic T cells and causes interstitial as well as vascular damage. This cellular response is most commonly seen in the initial months after transplantation. Immunosuppression of T cells is highly effective at preventing or limiting the activation of the recipient immune system by the transplanted organ, usually eliminating acute rejection. In **acute humoral rejection,** B cells in recipients who have not previously been sensitized to alloantigens

TABLE 43-1 Modes of Immune Rejection

| | HYPERACUTE REJECTION | ACUTE REJECTION | CHRONIC REJECTION |
|---|---|---|---|
| **Mechanism** | Pre-formed antibodies react with donor antigen and activate complement | Cellular—Donor antigen activates recipient T cells<br>Humoral—Recipient generates antibody response to donor antigen | Unknown, but believed to be caused by chronic inflammation resulting from T cell response to donor antigen |
| **Time Course** | Minutes to hours | Weeks to months | Months to years |
| **How Suppressed** | Matching donor and recipient blood types | Immunosuppression | Currently cannot be suppressed |

present in the transplanted organ produce antibodies to donor antigens over a period of 7–10 days unless the recipient is immunosuppressed following transplantation. The antibody response is typically directed against endothelial cells and thus is also known as **acute vascular rejection.** Even with immunosuppression, however, episodes of acute rejection can occur months or even years after transplantation.

### Chronic Rejection

**Chronic rejection** is believed to be humoral and cellular in nature and does not occur until months or years after transplantation. Because hyperacute and acute rejection are generally well controlled by donor/recipient matching and immunosuppression, chronic rejection is now the most common lifespan-limiting pathology of organ transplants.

Chronic rejection is believed to result from chronic inflammation caused by T cell response to donor antigen. Once these T cells are activated, they release cytokines that recruit macrophages into the graft. The macrophages induce chronic inflammation that leads to intimal proliferation of the vasculature and scarring of the graft tissue. The chronic changes eventually lead to irreversible organ failure. Other contributing nonimmune factors can include ischemic/reperfusion injury and infection.

No effective treatment regimens to eliminate chronic rejection are now available; however, it is believed that several experimental therapies have a reasonable chance of reducing chronic rejection. The development of tolerance through elimination of costimulation (see below) has shown promise in this area.

## Graft Versus Host Disease

In leukemia or primary immunodeficiency, bone marrow transplantation is a successful means of restoring immune function to the patient after his or her marrow has been destroyed by aggressive chemotherapy or radiation treatment. A major complication of bone marrow transplants is **graft versus host disease** (GVHD), an alloimmune reaction that occurs when transplanted immune cells attack the cells of the recipient. This reaction, which presents as a severe inflammatory disease, with rash, pneumonitis, and diarrhea, can often be ameliorated by removing T cells from the donor bone marrow before transplantation. Generally considered harmful to the recipient, GVHD can be beneficial when donor immune cells attack recipient tumor cells that have survived chemotherapy (in the case of leukemia, this is called the graft versus leukemia effect, or GVL). Therefore, although removing donor T cells from the "graft" reduces the risk of GVHD, this may not be the best option for marrow transplants used in antineoplastic therapy. GVHD is treated using immunosuppressive agents described in the pharmacology section of this chapter.

# AUTOIMMUNITY

Autoimmune diseases are caused by an attack by the host immune system on its own tissues. When the immune system mistakes self antigen for foreign, the response is a chronic inflammation.

Autoimmune diseases result from a breakdown of self-tolerance, both central and peripheral. **Central tolerance** refers to the specific clonal deletion of autoreactive T and B cells during their development from precursor cells in the thymus and bone marrow. Central tolerance ensures that the majority of immature autoreactive T and B cells do not develop into self-reactive clones. The thymus and bone marrow, however, do not express every antigen present in the body; a number of proteins are expressed only in specific tissues. For this reason, **peripheral tolerance** is also important. Peripheral tolerance can result from deletion of autoreactive T cells by Fas–Fas ligand-mediated apoptosis, activation of T suppressor cells, or induction of T cell anergy due to antigen presentation in the absence of costimulatory molecules.

Although breakdown in tolerance lies at the center of every autoimmune disease, the inciting stimulus leading to loss of tolerance is, in many cases, unknown. Genetic factors may play a role, with the presence of certain MHC molecules predisposing T cells to the loss of self-tolerance. For example, HLA-B27 is causally related to many forms of autoimmune spondylitis. Several other autoimmune diseases are linked with specific HLA loci, supporting an association, if not a causal role, for genetic predisposition to autoimmunity. Molecular mimicry, whereby epitopes from infectious agents are similar to the body's own antigens, can also lead to a breakdown of tolerance and may be the mechanism underlying poststreptococcal glomerulonephritis. A number of other processes, including failure of T-cell apoptosis, polyclonal lymphocyte activation, and exposure of cryptic self-antigens, have also been hypothesized to lead to autoimmunity. The details of these mechanisms are beyond the scope of this book; however, the end result of each is a *failure of self-tolerance.*

Once self-tolerance has been compromised, the specific expression of autoimmunity may take one of three general forms (Table 43-2). Those diseases that manifest as antibodies to a specific antigen, such as Goodpasture's syndrome, result from antibody-dependent opsonization of and cytotoxicity to the target organ. Some autoimmune vasculitis syndromes, by comparison, result in large circulating antibody–antigen complexes that deposit in vessels, resulting in inflammation and injury to the vessel, as in cryoglobulinemia. Finally, T cell-mediated diseases are caused by cytotoxic T cells that are specific to a self-antigen, resulting in destruction of a specific tissue expressing that antigen, such as occurs with pancreatic beta cells in insulin-dependent (Type 1) diabetes mellitus.

The pharmacologic therapy for autoimmune diseases does not yet match the exquisite specificity of the offending biological process. Most current pharmacologic agents cause generalized immunosuppression rather than target the specific pathophysiology. Understanding the molecular pathways leading to autoimmune diseases should reveal new pharmacologic targets that can be used to suppress the specific autoimmune response before an autoimmune condition arises.

# PHARMACOLOGIC CLASSES AND AGENTS

Pharmacologic suppression of the immune system utilizes seven mechanistic approaches (Fig. 43-1):

1. inhibition of gene expression to modulate inflammatory responses

**TABLE 43-2** Representative Examples of Autoimmune Diseases, Categorized by Type of Tissue Damage

ANTIBODY TO SELF-ANTIGEN

| SYNDROME | AUTOANTIGEN | CONSEQUENCE |
|---|---|---|
| Autoimmune hemolytic anemia | Rh blood group antigens | Destruction of red blood cells |
| Autoimmune thrombocytopenic purpura | Platelet GpIIb:IIIa | Excessive bleeding |
| Goodpasture's syndrome | Renal glomerular basement membrane collagen type IV | Glomerulonephritis, pulmonary hemorrhage |
| Pemphigus vulgaris | Epidermal cadherin | Blistering of skin |
| Acute rheumatic fever | Streptococcal cell wall antigens that cross-react with cardiac muscle | Arthritis, myocarditis |

IMMUNE-COMPLEX DISEASE

| SYNDROME | AUTOANTIGEN | CONSEQUENCE |
|---|---|---|
| Mixed essential cryoglobulinemia | Rheumatoid factor IgG complexes | Systemic vasculitis |
| Systemic lupus erythematosus | DNA, histones, ribosomes, snRNP, scRNP | Glomerulonephritis, vasculitis, arthritis |

T CELL-MEDIATED DISEASE

| SYNDROME | AUTOANTIGEN | CONSEQUENCE |
|---|---|---|
| Insulin-dependent diabetes mellitus | Pancreatic β cell antigen | β cell destruction, diabetes mellitus (Type 1) |
| Rheumatoid arthritis | Unknown—possible synovial joint antigen | Joint inflammation and destruction |
| Experimental autoimmune encephalitis, multiple sclerosis | Myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein | Brain invasion by CD4 T cells, variety of CNS deficits |

2. cytotoxic agents to deplete expanding lymphocyte populations
3. inhibition of lymphocyte signaling to block activation and expansion of lymphocytes
4. neutralization of cytokines essential for mediating the immune response
5. use of antibodies to deplete the immune system of specific cells
6. blockade of costimulation to induce anergy
7. blockade of cell adhesion to prevent localization of inflammatory cells

Some approaches are more specific than others, and lack of specificity often leads to toxicity. All of the drugs in this chapter target mediators important for adaptive immunity and either directly or indirectly prevent immune-mediated damage.

## INHIBITORS OF GENE EXPRESSION

### Glucocorticoids

**Glucocorticoids** have broad anti-inflammatory effects. The intimate relationship between cortisol and the immune system is discussed in Chapter 26, Pharmacology of the Adrenal Cortex. Briefly, glucocorticoids are steroid hormones that exert their physiologic actions by binding to the cytosolic glucocorticoid receptor. This glucocorticoid–glucocorticoid receptor complex then diffuses into the nucleus and binds to glucocorticoid response elements (GREs) in the promoter region of specific genes, either upregulating or downregulating gene expression.

Glucocorticoids downregulate expression of many inflammatory mediators, including key cytokines such as TNF-α, IL-1, and IL-4. The role of glucocorticoids in suppressing phospholipase A2 action is discussed in Chapter 40, Pharmacology of Eicosanoids. The overall effect of glucocorticoid administration is profoundly anti-inflammatory and immunosuppressive, explaining the use of glucocorticoids for the treatment of numerous inflammatory diseases, such as rheumatoid arthritis and transplant rejection.

Long-term glucocorticoid administration has important adverse effects. *Diabetes, reduced resistance to infection, osteoporosis, cataracts, increased appetite leading to weight gain, hypertension and its sequelae, as well as the masking of inflammation* must be closely monitored in patients receiving corticosteroids. *Abrupt cessation of glucocorticoid therapy can result in acute adrenal insufficiency* because the hypothalamus and pituitary gland require a number of weeks to re-establish adequate ACTH production. During this time, the underlying disease can worsen because of disinhibition of the immune

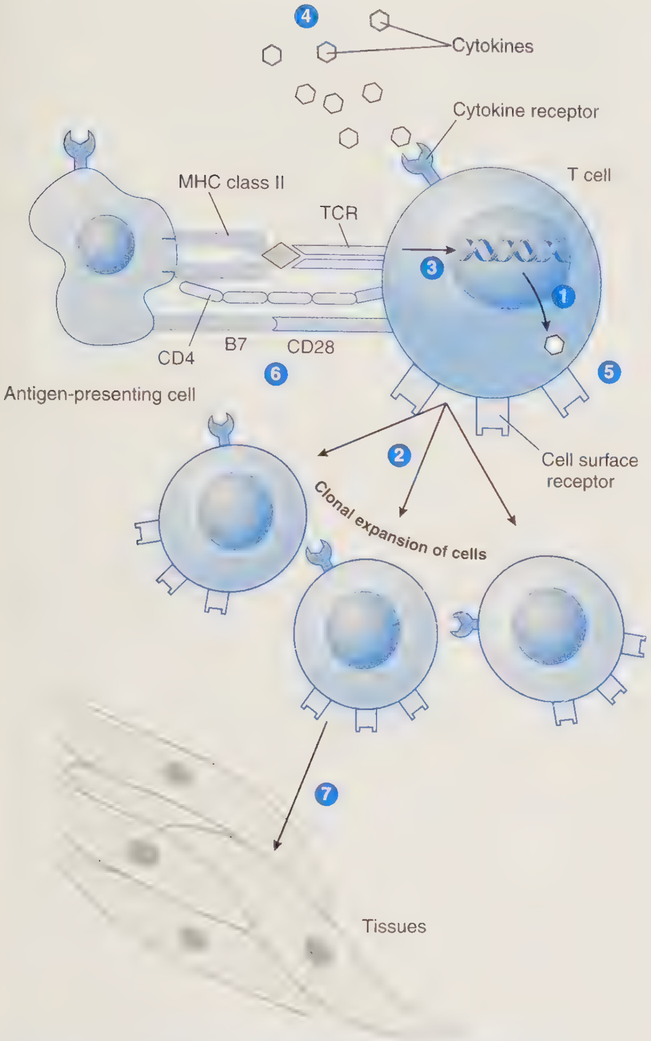


**Figure 43-1. Overview of Mechanisms of Pharmacologic Immunosuppression.** The molecular mechanisms by which T cells are activated and function provide seven major points for pharmacologic intervention by immunosuppressive agents. These include: (1) inhibition of gene expression; (2) selective attack on clonally expanding lymphocyte populations; (3) inhibition of intracellular signaling; (4) neutralization of cytokines required for T cell stimulation; (5) inhibition of cell surface receptor function and/or induction of lymphocyte cytotoxicity by antilymphocyte antibodies; (6) inhibition of costimulation by antigen-presenting cells; and (7) inhibition of lymphocyte–target cell interactions.

system. To prevent this complication, glucocorticoid dosage should be tapered slowly during termination of therapy.

## CYTOTOXIC AGENTS

Cytotoxic agents are used for immunosuppression as well as for antineoplastic chemotherapy. Two classes of cytotoxic agents, **antimetabolites** and **alkylating agents,** are commonly used as immunosuppressants. Antimetabolites are molecules that compete with essential components of metabolic processes and inhibit those processes. Alkylating agents interfere with DNA replication and gene expression by adding alkyl groups to DNA. The goal in both antineoplastic chemotherapy and immunosuppression is the elimination of undesirable cells.

### Antimetabolites

For many years, antimetabolites have been a mainstay of immunosuppression. Their powerful effect on immunity comes with many side effects related to a lack of specificity. The older antimetabolites, such as azathioprine and methotrexate, affect all rapidly dividing cells and can have devastating effects on the lining of gut as well as their intended targets. Newer antimetabolites, such as mycophenolate mofetil and leflunomide, can be used therapeutically at lower doses and cause fewer adverse effects. Mycophenolate mofetil may also be more specific to immune cells, further reducing toxicities. Antimetabolites typically affect both cell-mediated and humoral immunity, leaving patients more susceptible to secondary infection than would occur if only one of these systems were affected.

Because antimetabolites are widely used in the treatment of neoplasias, the underlying principles of their mechanisms are described in Chapter 31, Pharmacology of Nucleotide Synthesis. The specific molecular aspects of their mechanisms of action are briefly described below; Chapter 31, however, gives a more detailed description of the mechanisms of action of these agents.

#### *Azathioprine*

**Azathioprine** (AZA) was the first drug to be used for the suppression of the immune system following transplantation and remains a mainstay. Azathioprine is a prodrug of the purine analogue 6-mercaptopurine (6-MP), which is slowly released as AZA reacts with such sulfhydryl compounds as glutathione (Fig. 43-2). *The slow release of 6-MP from AZA favors*

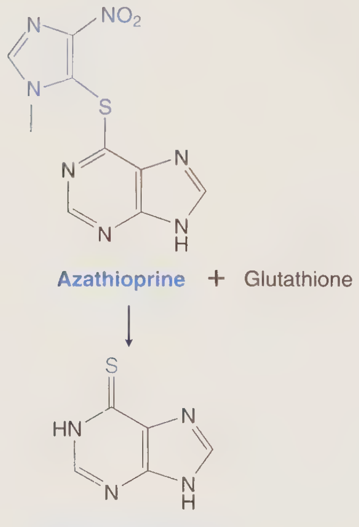


**Figure 43-2. Formation of Mercaptopurine from Azathioprine.** Azathioprine is a prodrug form of the antimetabolite 6-mercaptopurine. Mercaptopurine is formed by the cleavage of azathioprine in a nonenzymatic reaction with glutathione. Although mercaptopurine can also be used directly as a cytotoxic agent, azathioprine has a slower onset and longer duration of action than mercaptopurine.

*immunosuppression, while 6-MP itself is more useful as an antineoplastic drug.*

AZA prolongs organ graft survival. However, for kidney allograft transplantation, AZA has recently been shown to be less efficacious than mycophenolate mofetil in improving long-term graft survival. AZA is also used as an immunosuppressant for patients with inflammatory bowel disease.

### Methotrexate

**Methotrexate** (MTX) is a folate analogue used since the 1950s to treat malignancies. Since that time, methotrexate has become an extremely versatile drug used in a wide variety of immune-mediated diseases, including rheumatoid arthritis and psoriasis. MTX is also used for the prevention of GVHD.

In addition to its cytotoxic activity, MTX has independent anti-inflammatory activity. In the treatment of rheumatoid arthritis, administration of MTX and folate together is as effective as MTX alone, indicating that the anti-inflammatory properties of MTX may not wholly be caused by the effects on folate metabolism. The mechanism by which MTX mediates its anti-inflammatory effect is uncertain; however, it seems that MTX increases adenosine levels. Adenosine is a potent endogenous anti-inflammatory agent that inhibits neutrophil superoxide anion generation, neutrophil adhesion, and phagocytosis of immunoglobulin-coated particles.

MTX has also been shown to cause apoptosis of activated, but not of resting, CD4 and CD8 T cells. Other immunosuppressive agents, including 5-fluorouracil, 6-mercaptopurine, and mycophenolic acid have also been shown to promote apoptosis. MTX shows anti-inflammatory, anti-cell-mediated, and antihumoral effects, possibly explaining why MTX is such a versatile drug.

### Mycophenolic Acid and Mycophenolate Mofetil

**Mycophenolic acid** (MPA) is an inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the formation of guanosine. MPA has low oral bioavailability and is usually administered as a prodrug, **mycophenolate mofetil** (MMF) (Fig. 43-3), which has much higher oral bioavailability than MPA and is readily converted to MPA. Because of its high specificity and profound effect on lymphocytes, it appears that MMF will become an important drug in the treatment of immune-mediated disease.

MPA and MMF both act primarily on lymphocytes. Two main factors contribute to this specificity. First, lymphocytes are dependent on the de novo pathway of purine synthesis, as discussed in Chapter 31; whereas, most other tissues rely heavily on the salvage pathway. Because IMPDH is required for de novo synthesis of guanosine nucleotides, but not for the salvage pathway, MPA only affects such cells as lymphocytes that rely on de novo purine synthesis.

In addition, IMPDH is found in two isoforms, type I and type II. MPA preferentially inhibits type II IMPDH, the isoform that is highly expressed in lymphocytes. This leads to selectivity against T and B cells, with low toxicity to other cells.

The inhibition of IMPDH by MPA leads to reduced intracellular levels of guanosine nucleotides and elevated adenosine levels, leading to many effects on lymphocyte activation and activity (Table 43-3). MPA has a cytostatic effect on lymphocytes, but it can also induce apoptosis of activated T cells, leading to the elimination of reactive lines of proliferative cells. Because guanosine is required for some glycosylation reactions, the reduced level of guanosine nucleotides leads to reduced expression of adhesion molecules that recruit several immune system participants. Guanosine is a precursor of tetrahydrobiopterin, BH4, which regulates inducible nitric oxide synthase (NOS). Reduced guanosine levels cause less NO production by neutrophils. The constitutive NOS, which controls vascular tone and is regulated by $Ca^{2+}$ and calmodulin, is not affected, again demonstrating the considerable specificity of MPA.

Human studies comparing MMF and AZA have shown MMF to be more efficacious in preventing acute rejection of kidney transplants. In addition to inhibiting lymphocyte proliferation, MMF limits the smooth muscle cell proliferation seen in chronic rejection. Animal models also show that chronic rejection is reduced more effectively in recipients treated with MMF than those treated with AZA or cyclosporine.

MMF is also efficacious in the treatment of rheumatoid arthritis. Reduced levels of rheumatoid factor, immunoglobulin, and T cells occur in response to treatment with MMF. There have also been isolated reports of successful treatment of myasthenia gravis, psoriasis, autoimmune hemolytic anemia, and inflammatory bowel disease with MMF.

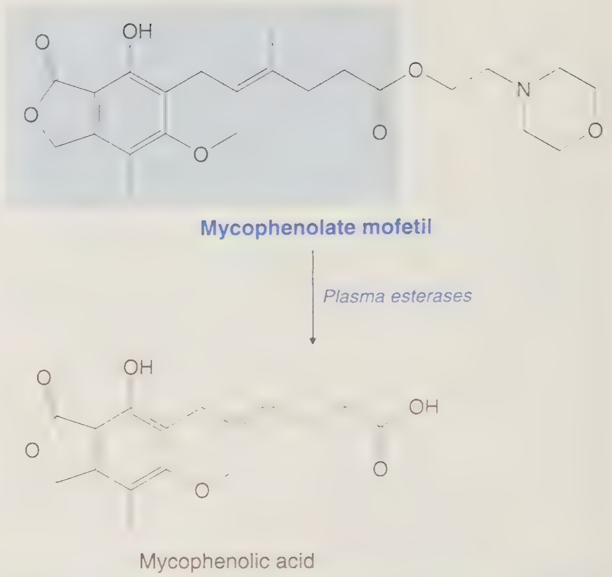


**Figure 43-3. Mycophenolic Acid and Mycophenolate Mofetil.** Mycophenolate mofetil (MMF) has higher oral bioavailability than mycophenolic acid (MPA). Orally administered mycophenolate mofetil is absorbed into the circulation, where plasma esterases rapidly cleave the ester bond to yield mycophenolic acid. Both agents inhibit inosine monophosphate dehydrogenase type II (IMPDH II), an enzyme crucial for de novo synthesis of guanosine. Because of its higher oral bioavailability, MMF is typically used.

**TABLE 43-3** Immune System Effects of Mycophenolate Mofetil

| |
|---|
| Cytostatic effect on lymphocytes |
| Reduced expression of adhesion molecules |
| Reduced nitric oxide synthesis by neutrophils |
| Limits smooth muscle proliferation occurring in chronic allograft rejection |

MMF may have antimicrobial activity. Because bacterial IMPDH is not homologous to human IMPDH, MMF does not have antibacterial effects; however, fungi (including *Pneumocystis carinii*) and certain parasites are affected by MMF in animal models. MMF also reduces the replication of viruses that infect lymphocytes, including HIV (T cells) and Epstein–Barr virus (EBV) (B cells), possibly by limiting transcription in infected cells due to reduced guanosine nucleotide levels. In addition, cells infected with EBV shift from the type I to the type II isoform of IMPDH, thereby making EBV-infected cells targets for MMF.

### Leflunomide

Activated lymphocytes must proliferate and synthesize large quantities of cytokines and other effector molecules, processes requiring increased DNA and RNA synthesis. Therefore, agents that reduce intracellular nucleotides will have effects on these activated cells. **Leflunomide** is an inhibitor of pyrimidine synthesis, specifically blocking synthesis of UMP by inhibiting dihydroorotate dehydrogenase (DHOD). DHOD is a key enzyme in the synthesis of UMP (Fig. 43-4), which is essential for the synthesis of all pyrimidines. (See Chapter 31 for a review of pyrimidine synthesis.) Experimentally, leflunomide has been shown to be most effective in reducing B cell populations, but a significant T cell effect has also been observed.

Currently approved for rheumatoid arthritis, leflunomide has shown significant efficacy in the treatment of other diseases of immunity, including Wegener's granulomatosis, systemic lupus erythematosus, and myasthenia gravis. Leflunomide prolongs transplant graft survival and limits GVHD in animal models.

The most significant adverse effects of leflunomide are diarrhea and reversible alopecia. Leflunomide undergoes significant enterohepatic circulation, resulting in a prolonged pharmacologic effect. If leflunomide must be removed quickly from a patient's system, cholestyramine may be administered. Cholestyramine binds to bile acids, which interrupts enterohepatic recirculation and causes a "washout" of leflunomide.

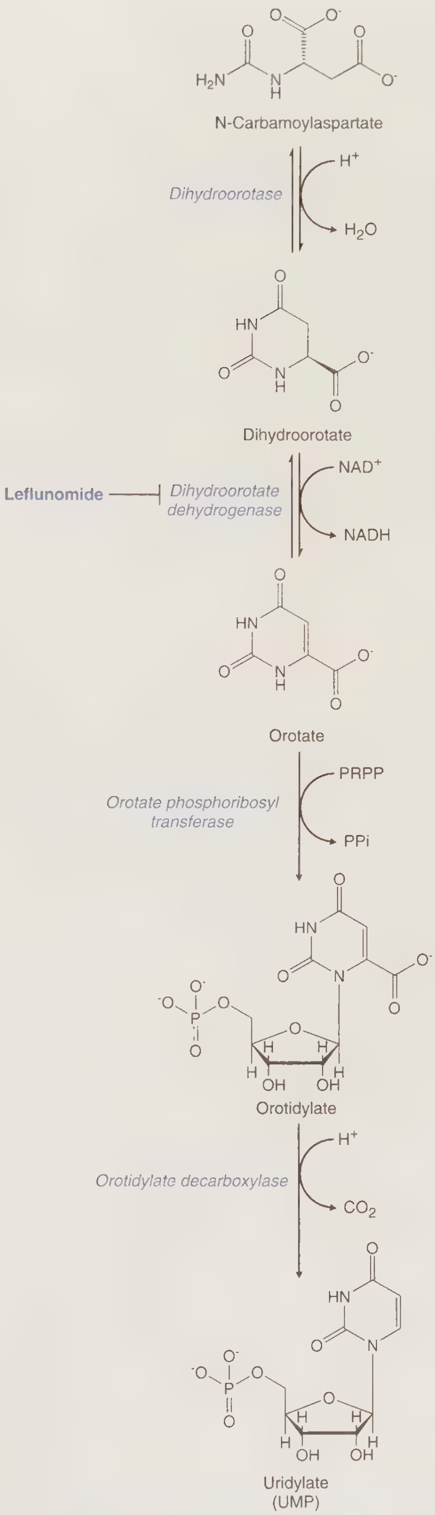


**Figure 43-4. Inhibition of Pyrimidine Synthesis by Leflunomide.** De novo pyrimidine synthesis depends on the oxidation of dihydroorotate to orotate, a reaction that is catalyzed by dihydroorotate dehydrogenase. Leflunomide inhibits dihydroorotate dehydrogenase and, thereby, inhibits pyrimidine synthesis. Because lymphocytes are dependent on de novo pyrimidine synthesis for cell replication and clonal expansion after immune cell activation, depletion of the pyrimidine pool inhibits lymphocyte expansion. Experimentally, leflunomide seems to inhibit preferentially the replication of B cells; the reason for this preferential action is unknown.

## Alkylating Agents

### Cyclophosphamide

**Cyclophosphamide** (Cy) is a highly toxic drug that alkylates DNA. The mechanism and uses of Cy are discussed extensively in Chapter 32, Pharmacology of DNA Replication and Mitosis; therefore, the discussion here is limited to Cy's utility in treating diseases of the immune system. Because Cy has a major effect on B cell proliferation and can enhance T cell responses, the use of Cy is limited to diseases of humoral immunity, particularly systemic lupus erythematosus. Another use being considered for Cy is in the suppression of antibody formation against xenotransplant grafts. Side effects of Cy are severe and widespread and include leukopenia, cardiotoxicity, alopecia, an increased risk of cancer because of mutagenicity, and especially bladder cancer because of production of a carcinogenic metabolite, **acrolein.** Acrolein can be detoxified by coadministration of mesna (a sulfhydryl-containing compound that neutralizes the reactive groups of acrolein) with Cy, when Cy is given by intravenous infusion.

# SPECIFIC LYMPHOCYTE SIGNALING INHIBITORS

## Cyclosporine and Tacrolimus

The discovery in 1976 that **cyclosporine** (CsA, also referred to as cyclosporin A) is a specific inhibitor of T cell-mediated immunity enabled widespread whole-organ transplantation. In fact, CsA made heart transplantation a legitimate alternative in the treatment of end-stage heart failure. CsA is a decapeptide isolated from a soil fungus, *Tolypocladium inflatum.* CsA inhibits the production of IL-2 by activated T cells. IL-2 acts in an autocrine and paracrine manner to cause activation and proliferation of additional T cells.

The action of CsA is mediated by blockade of T cell signaling (Fig. 43-5). Activated T cells increase production of IL-2 via a pathway that begins with dephosphorylation of a cytoplasmic transcription factor, **NFAT** (nuclear factor of activated T cells) by the phosphatase **calcineurin.** When dephosphorylated, NFAT moves to the nucleus and enhances transcription of the IL-2 gene. CsA binds to cyclophilin and the CsA-cyclophilin complex binds to and inhibits the phosphatase activity of calcineurin. By inhibiting calcineurin-mediated dephosphorylation of NFAT, CsA prevents the movement of NFAT to the nucleus, thereby suppressing production of IL-2.

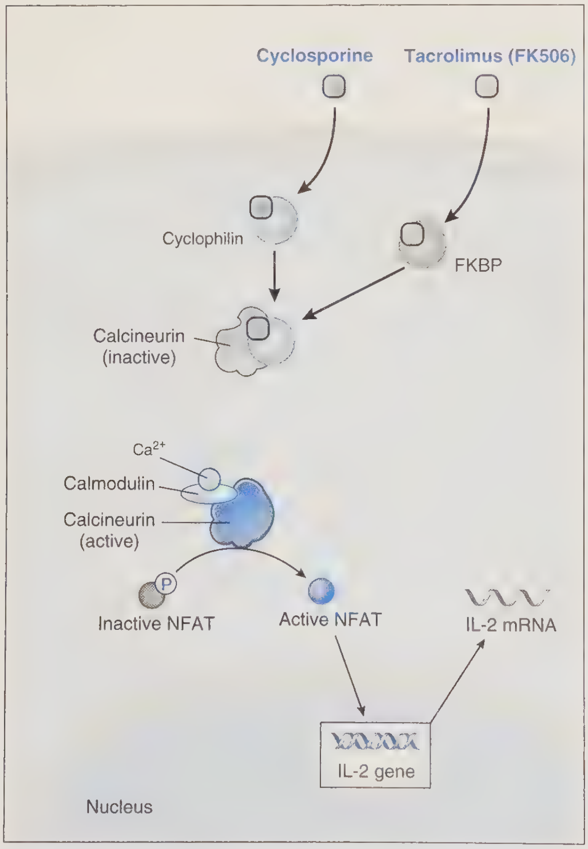


**Figure 43-5. Mechanisms of Action of Cyclosporine and Tacrolimus.** The actions of cyclosporine and tacrolimus (also known as FK506) are mediated by blockade of intracellular T cell signaling. In normal T cell signaling (bottom of figure), stimulation of T cells increases the level of intracellular calcium, and Ca$^{2+}$/calmodulin activates the calcineurin-mediated dephosphorylation of the cytoplasmic transcription factor NFAT. Activated NFAT migrates into the nucleus, where it induces IL-2 gene transcription. Cyclosporine and tacrolimus cross the plasma membrane and bind to the cytoplasmic immunophilins cyclophilin and FK binding protein (FKBP), respectively (top of figure). Both the cyclosporine–cyclophilin and tacrolimus–FKBP complexes bind to calcineurin, preventing the activation of calcineurin phosphatase activity by Ca$^{2+}$/calmodulin.

The usefulness of CsA is limited by severe side effects, which include *nephrotoxicity, neurotoxicity and hepatotoxicity.* Cyclosporine nephrotoxicity is the probable reason for Mrs. White's chronic renal insufficiency. CsA increases production of transforming growth factor-β (TGF-β), which causes cells to increase production of extracellular matrix, resulting in interstitial fibrosis. Despite these side effects, CsA is an essential part of most clinical immunosuppressive regimens.

**Tacrolimus** (also known as FK506) is a more potent immunosuppressant drug with a different structure from CsA; however, it acts by a similar mechanism (Fig. 43-5). Tacrolimus is a macrocyclic triene isolated from the bacterium, *Streptomyces tsukubaensis.* Tacrolimus binds to FK-binding proteins (**FKBP**) and the tacrolimus:FKBP complex inhibits calcineurin. In vitro, tacrolimus inhibits IL-3, IL-4, γ-IFN, and TNF-α activity and appears to inhibit cell-mediated immunity without suppressing B cell or NK cell function. It is generally 50–100 times more potent than CsA but, like CsA, it is nephrotoxic.

Tacrolimus is approved as an immunosuppressant for transplantation. It is also used by topical application for the treatment of atopic eczema.

## Sirolimus

**Sirolimus,** also referred to as rapamycin, is a macrocyclic triene isolated from the fungus *Streptomyces hygroscopicus.* Although they are structurally similar and are both used to prevent and treat organ rejection, tacrolimus and sirolimus have different mechanisms of action. Both bind to FKBP, but the sirolimus:FKBP complex does not inhibit calcineurin—instead, it blocks IL-2 receptor signaling (Fig. 43-6). Sirolimus:FKBP inhibits mTOR (molecular target of rapamycin), thereby inhibiting protein synthesis. mTOR is a kinase that phosphorylates p70 S6 kinase, which regulates transcription, and PHAS-1, a repressor of mRNA translation. Inhibition of protein synthesis by sirolimus arrests cell division in the G1 phase (Fig. 43-6). Major adverse effects of sirolimus include hyperlipidemia, leukopenia, and thrombocytopenia. Notably, however, the nephrotoxicity associated with CsA and FK506 is not observed with sirolimus.

Recently, **sirolimus-eluting stents** have been approved for use in the treatment of coronary artery disease. In this unique drug delivery system, sirolimus elutes from stents during the first few weeks after stent placement, locally inhibits proliferation of coronary artery smooth muscle cells and, thereby, significantly reduces the rate of in-stent restenosis that results from neointimal proliferation of vascular smooth muscle cells.

# CYTOKINE INHIBITION

Cytokines are critical signaling mediators in immune function. Cytokines are also pleiotropic; that is, they may exert

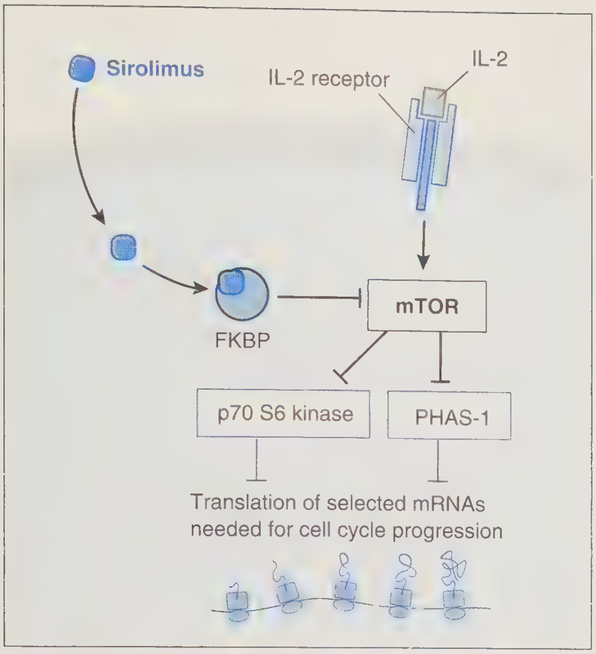

Sirolimus
IL-2 receptor
IL-2
FKBP
mTOR
p70 S6 kinase
PHAS-1
Translation of selected mRNAs needed for cell cycle progression

**Figure 43-6. Mechanism of Action of Sirolimus.** IL-2 receptor signal transduction involves a complex set of protein–protein interactions that lead to increased translation of selected mRNAs encoding proteins required for T cell division. Specifically, IL-2 receptor activation initiates an intracellular signaling cascade that leads to phosphorylation of the molecular target of rapamycin (mTOR). mTOR is a kinase that phosphorylates and, thereby, inactivates PHAS-1 and p70 S6 kinase, which are inhibitors of translation. The net effect of mTOR activation is to increase protein translation (by inhibiting an inhibitor) and, therefore, to promote the transition from G1 to S phase of the cell cycle. Sirolimus (also known as rapamycin) crosses the plasma membrane and binds to intracellular FK binding protein (FKBP). The sirolimus-FKBP complex inhibits mTOR, thereby inhibiting translation and causing T cells to arrest in the G1 phase.

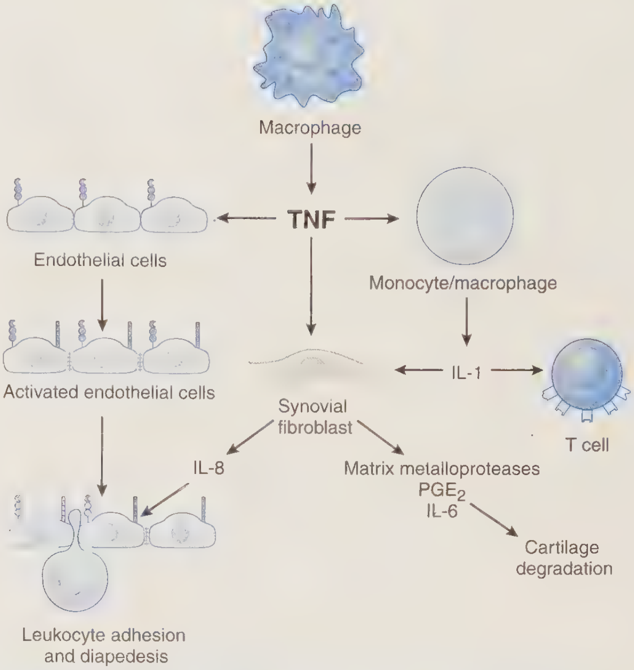


**Figure 43-7. Proposed Roles for Tumor Necrosis Factor in Rheumatoid Arthritis.** Tumor necrosis factor (TNF) is secreted by activated macrophages in an affected joint, where this cytokine has multiple proinflammatory effects. First, TNF activates endothelial cells to upregulate their expression of cell surface adhesion molecules (shown as projections on endothelial cells) and undergo other phenotypic changes that promote leukocyte adhesion and diapedesis. Second, TNF has a positive feedback effect on nearby monocytes and macrophages, promoting their secretion of such cytokines as IL-1. IL-1 activates T cells (among other functions), and the combination of IL-1 and TNF stimulates synovial fibroblasts to increase their expression of matrix metalloproteases, prostaglandins (especially PGE$_2$), and cytokines (such as IL-6) that degrade the joint cartilage. Synovial fibroblasts also secrete IL-8, which promotes neutrophil diapedesis.

different effects depending on the target cell and overall cytokine milieu. For this reason, pharmacologic uses of cytokines or cytokine inhibitors may have unpredictable effects. Anticytokine therapy is still in its infancy, with a number of new drugs that inhibit proinflammatory cytokines under development.

## TNF-α Inhibitors

**Tumor necrosis factor-α (TNF-α)** is a cytokine central to many aspects of the inflammatory response. Macrophages, mast cells, and activated $T_H$ cells (especially $T_{H1}$ cells) secrete TNF-α. TNF-α stimulates macrophages to produce cytotoxic metabolites, thereby increasing phagocytic killing activity. TNF-α also stimulates production of acute phase proteins, has pyrogenic effects, and fosters local containment of the inflammatory response. Some of these effects are indirect and are mediated by other cytokines induced by TNF-α.

TNF-α has been implicated in numerous autoimmune diseases. Rheumatoid arthritis and Crohn's disease are two such disorders in which inhibition of TNF-α has demonstrated therapeutic efficacy. Recent models of rheumatoid arthritis postulate a central role for TNF-α in disease pathogenesis (Fig. 43-7). Although the initial stimulus for joint inflammation is still debated, it is thought that macrophages in a diseased joint secrete TNF-α, which activates endothelial cells, other monocytes, and synovial fibroblasts. Activated endothelial cells upregulate adhesion molecule expression, resulting in localization of inflammatory cells and mediators in the joint. Increased monocyte activation has a positive feedback effect on T cell and synovial fibroblast activation. Finally, activated synovial fibroblasts secrete interleukins, promoting additional leukocyte localization. The synovium hypertrophies, forming a pannus that leads to destruction of joint bone and cartilage and the characteristic deformity and pain of rheumatoid arthritis.

Crohn's disease is an inflammatory disease characterized by mucosal inflammation, usually in multiple areas, of the small and large intestine. Crohn's disease has an autoimmune component. T cells are activated inappropriately and drive a $T_{H1}$-mediated inflammatory response in the bowel mucosa. As in rheumatoid arthritis, TNF-α mediates many of the inflammatory components of Crohn's disease.

Three therapies interfering with TNF-α activity have been approved. **Etanercept** is a soluble TNF receptor dimer; **infliximab** is a partially humanized mouse antibody to human TNF-α; **adalimumab** is a fully humanized IgG1 antibody to

TNF-α (Fig. 43-8). Although all agents target TNF-α, etanercept is currently approved for rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis; infliximab is approved for both rheumatoid arthritis and Crohn's disease; and adalimumab is currently approved only for rheumatoid arthritis. Other indications are being investigated and off-label uses by physicians include psoriatic arthritis, HIV arthropathy, and such wasting syndromes as HIV and cardiac cachexia. Etanercept is somewhat less specific, binding to both TNF-α and TNF-β. Infliximab and adalimumab are TNF-α specific and do not bind TNF-β. The $F_C$ positions of the molecules may also have specific activity with respect to complement fixation and binding to $F_C$ receptors on effector cells.

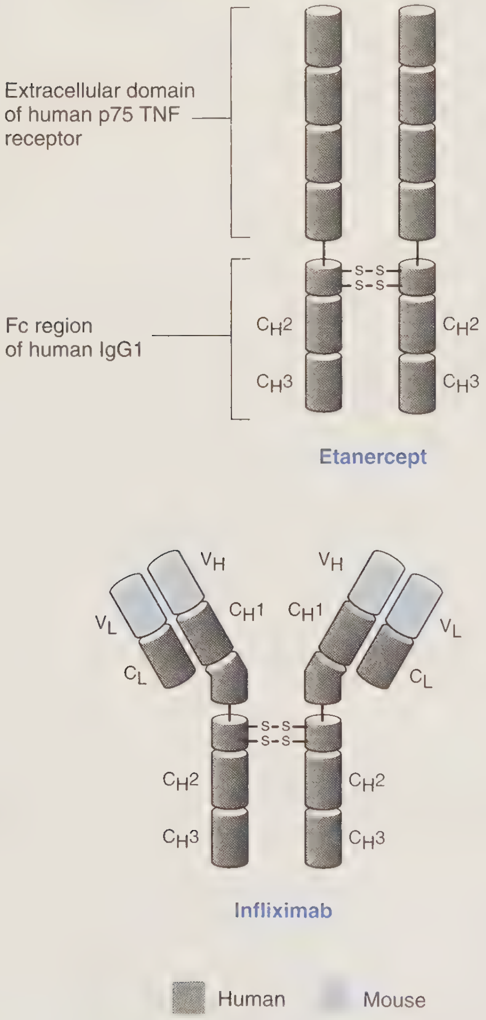


**Figure 43-8. Anti-TNF Agents.** Shown are the molecular domain organizations of etanercept and infliximab. Etanercept is the extracellular portion of the human TNF receptor fused to the Fc region of human IgG1. This "decoy" receptor binds TNF-α and TNF-β in the circulation, preventing the access of these cytokines to target tissues. Infliximab is a partially humanized monoclonal antibody against TNF-α. The variable heavy chain ($V_H$) and variable light chain ($V_L$) regions are derived from mouse antihuman sequences, while the remainder of the antibody (the constant regions, denoted by "C") is composed of human antibody sequences. This modification of the original mouse monoclonal anti-TNF-α antibody reduces the development of neutralizing antibodies against infliximab. Adalimumab (not shown), a fully humanized antibody to human TNF-α, has recently been developed.

It is important to recognize that, in both Crohn's disease and rheumatoid arthritis, high levels of TNF-α are likely attributable to pathophysiologic processes. Treatment with etanercept, infliximab or adalimumab improves disease symptoms but does not reverse the underlying pathophysiology. Therefore, the long-term efficacy of these therapies may be limited. In addition, etanercept, infliximab, and adalimumab are proteins and must be administered parenterally. Orally active inhibitors of TNF-α, such as thalidomide and inhibitors of TNF-α converting enzyme (TACE), are under investigation.

A number of important side effects must be considered when administering TNF inhibitors. All patients should undergo screening for tuberculosis before initiating therapy because of a greatly increased risk of reactivating latent tuberculosis. Any patient developing an infection while taking a TNF-α inhibitor should undergo evaluation and aggressive antibiotic treatment. Epidemiologic surveillance has also suggested that there may be an increased risk of demyelinating disease with anti-TNF therapy, although it has not yet been determined if there is a causal relationship.

### IL-1 Inhibitors

**Interleukin-1 (IL-1)** is an ancient cytokine, found in both vertebrates and invertebrates, that serves as a bridge between innate and adaptive immunity. There are two forms of IL-1, IL-1α and IL-1β, encoded on different genes. Most IL-1 is generated by activated mononuclear cells. IL-1 stimulates production of IL-6, enhances transcription of adhesion molecules, and can stimulate cell proliferation. Modulation of IL-1 activity in vivo is accomplished in part by an endogenous IL-1 receptor antagonist (IL-1ra). A recombinant form of IL-1ra, given the generic name **anakinra,** has been developed as an anti-inflammatory therapy and has been tested in rheumatoid arthritis. Modest clinical effects on pain and swelling were observed in clinical trials, and a significant reduction in bony erosions, possibly because of blockade of IL-1-induced metalloproteinase release from synovial cells and decreased osteoclast production. Anakinra was recently approved by the FDA for retarding the development of bony erosions in rheumatoid arthritis.

## ANTIBODIES

The use of antibodies to deplete the immune system of reactive cells is an effective therapy for a number of autoimmune and transplant rejection conditions. When the adaptive immune system recognizes a foreign epitope, an immunologic response is mounted by clonal expansion of cells specifically reactive to that target. Treatment with exogenous antibodies against cell-surface molecules present on immune cells can preferentially deplete the immune system of these reactive cells.

### Polyclonal Antibodies

#### *Anti-Thymocyte Globulin*

**Anti-thymocyte globulin (ATG)** is a preparation of antibodies against thymocytes prepared by injecting rabbits with human thymocytes. The antibodies produced are polyclonal, and probably target many epitopes on human T cells. Because it targets essentially all T cells, ATG treatment can result in a broad immunosuppression that can lead to infection. ATG is approved for treatment of acute renal transplant rejection and is administered intravenously over 1–2 weeks.

Common side effects of ATG include fever and headache, called the **cytokine release syndrome.** This side effect, common to many antibody drugs that target lymphocytes, results from initial activation of T cells and release of cytokines before T cell clearance by macrophages. The cytokine release syndrome is common following the first few doses of T cell antibody therapy, and the symptoms dissipate as T cells are eliminated. However, administration of successive doses can be complicated by development of antibodies against rabbit-specific epitopes on the administered immunoglobulins.

## Monoclonal Antibodies

### OKT3

**OKT3** (muromonab-CD3, anti-CD3) is a mouse monoclonal antibody against human CD3, one of the cell membrane signaling molecules important for activation of the T cell receptor. CD3 is specifically expressed on T cells (both $CD4^+$ and $CD8^+$ cells). Treatment with OKT3 depletes the available pool of T cells via antibody-mediated activation of complement and clearance of immune complexes. OKT3 is currently approved for treatment of acute renal transplant rejection and is considered a second-line agent for use when CsA or glucocorticoids fail.

Because OKT3 targets all T cells, treatment can result in a profound but transient immunosuppression, but T cell levels return to normal within a week after discontinuing therapy. Because OKT3 binds to CD3 and CD3 is important for T cell activation, OKT3 therapy can sometimes broadly activate T cells. This results in the cytokine release syndrome. In the introductory case, the fever, myalgias, nausea, and diarrhea after administration of OKT3 as well as Mrs. White's complaint that "this OKT3 makes me sleepy" were likely manifestations of the cytokine release syndrome.

Another limitation of OKT3 therapy is that it is a mouse-anti-human antibody. Because it is foreign, treatment can result in antibodies against the mouse-specific regions of OKT3. This is why Mrs. White was tested for the anti-OKT3 antibodies when she returned to the hospital in December 1990. The presence of antibodies reduces drug efficacy by sequestering OKT3 before it can exert its desired effect. To solve this clinical problem, a new approach is to **humanize** antibodies. To humanize antibodies, the portions of the protein not involved in binding to the antigen are changed to the corresponding human sequences. Antibodies can be partially or fully humanized, depending on the extent of these changes. Humanization limits the production of human antibodies against the pharmacologic agent, raising therapeutic efficiency of the antibody and also allowing long-term use.

### Anti-CD25 mAb

**Daclizumab** and **basiliximab** are anti-CD25 antibodies. CD25 is the high-affinity IL-2 receptor. IL-2 mediates early steps in T cell activation. CD25 is expressed only on activated T cells, which makes anti-CD25 antibody specific for only those T cells activated by an MHC-antigenic stimulus.

Daclizumab is administered prophylactically in renal transplants to inhibit acute organ rejection. It is also used as part of general immunosuppressive regimens after organ transplantation. It is typically administered in a five-dose regimen, with administration immediately after transplantation and then four additional doses at 2-week intervals. This type of dosing regimen, where drug administration ceases at some point shortly after transplantation, is referred to as **induction therapy.**

### Anti-CD52 mAb

**Campath-1 (CD52)** is an antigen found on most lymphocytes and some precursors. An antibody to this antigen was tested in rheumatoid arthritis and found to cause a prolonged and sustained depletion of all T cells, often lasting years. This led to improvement of the arthritis symptoms; however, the sustained depletion of lymphocytes and concern about infections inhibited further study of this antibody in autoimmune conditions. This antibody, under the generic name **alemtuzumab,** has recently been approved as an adjunctive therapy in the treatment of B-cell chronic lymphocytic leukemia, a condition in which sustained suppression of the cells is desirable.

## INHIBITION OF COSTIMULATION

Although there are no approved drugs that inhibit costimulation of T cells, current research on tolerance induction has focused on the role of costimulatory molecules in lymphocyte activation. **Costimulation** refers to the paradigm that cells of the immune system typically require two signals for activation (Fig. 43-9). Signal 1 provides specificity, while signal 2 is permissive, signaling that an inflammatory response is appropriate. Regulation of costimulatory molecules may be a mechanism whereby the immune system limits the extent of an immune response. If antigen is presented without a coincident costimulatory signal, anergy results, whereby a cell becomes unreactive and will not respond to further antigenic stimuli. *Induction of anergy could lead to long-term acceptance of an organ graft or limit the extent of an autoimmune disease.*

For T cells, Signal 1 is mediated by the MHC:TCR interaction. Signal 2 is mediated predominantly by the interaction of CD28 of T cells with B7-1 or B7-2 on activated antigen-presenting cells. Resting T cells present CD28, which can bind either B7-1 or B7-2. B7-1 and B7-2 are not normally present on antigen-presenting cells, but their expression is increased during an inflammatory response mediated by the innate immune system. The lack of expression of B7 molecules during noninflammatory states may help to limit inappropriate adaptive immune responses. Expression of IL-2, T-cell activation, and clonal expansion of $T_H$ cells specific for that foreign epitope occur when a T cell receives both signal 1 and signal 2. Interestingly, activated T cells eventually downregulate CD28 expression and upregulate CTLA-4 expression. **CTLA-4,** like CD28, binds B7-1 and B7-2, but with much higher affinity than CD28. In contrast to the activating CD28 signal, interaction of CTLA-4 with B7-1 or B7-2 inhibits T cell proliferation. This also may be a physiologic mechanism for self-limitation of the immune response.

*Because engagement of signal 1 in the absence of signal 2 results in anergy, administration of a costimulation inhibitor could be an effective means for downregulating specific T cell populations.* A pharmacologic agent that blocks costimulation would preferentially inhibit only those T cells that are reactive. **CTLA-4 Ig** is the most studied molecule to block costimulation. CTLA-4 Ig consists of CTLA-4 fused to an

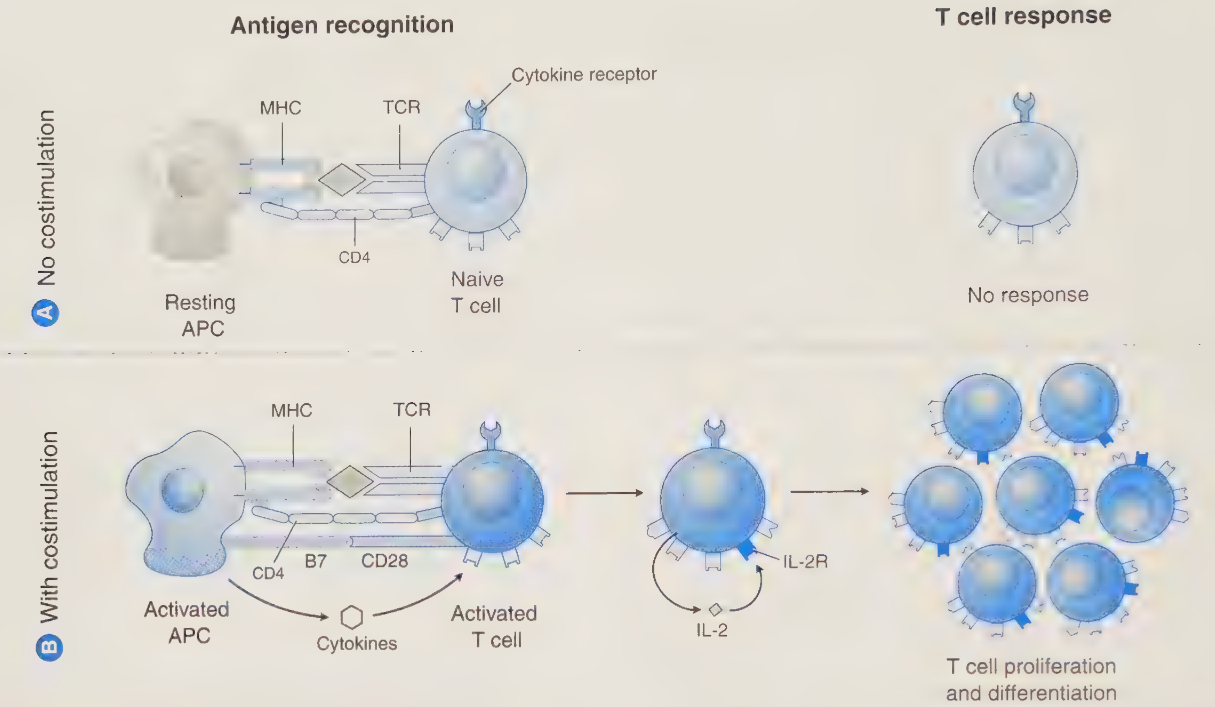

Antigen recognition
T cell response
A No costimulation
Cytokine receptor
MHC
TCR
CD4
Resting APC
Naive T cell
No response
B With costimulation
MHC
TCR
CD4
B7
CD28
Activated APC
Cytokines
Activated T cell
IL-2R
IL-2
T cell proliferation and differentiation

Figure 43-9. Costimulation in the T Cell Activation Pathway. Two signals are required for activation of a T cell response to antigen. **A.** If an antigen-presenting cell (APC) presents an antigen to a T cell in the absence of an appropriate costimulatory signal, the T cell does not respond, and may become anergic. **B.** If an APC presents both the antigen and a costimulatory molecule such as B7, the T cell proliferates and differentiates in response to the antigenic stimulus. Cytokines secreted by the activated APC (see Fig. 43-10) augment T cell activation.

IgG1 constant region. CTLA-4 Ig complexes with the surface B7 molecules of antigen presenting cells. When the antigen-presenting cell interacts with a T cell, MHC-TCR interaction occurs, but the complex of B7 with CTLA-4 prevents delivery of a costimulatory signal, and the T cell develops anergy or undergoes apoptosis. Experiments have demonstrated that administration of CTLA-4 Ig can prolong graft survival in animal transplantation models. CTLA-4 Ig is also under investigation in such autoimmune diseases as rheumatoid arthritis and systemic lupus erythematosus. Antibodies to B7-1 and B7-2 are also under investigation.

**CD40 ligand (CD40L)** is another target of costimulation. Activated T cells express CD40L (CD154). CD40 is expressed on antigen-presenting cells, including macrophages and B cells (Fig. 43-10). The CD40-CD40L interaction on T cells promotes B cell activation, isotype switching, and affinity maturation. Interaction of $T_H$ cell CD40L with macrophage CD40 promotes macrophage expression of B7-1 and B7-2. These molecules, as mentioned above, are crucial for costimulation of T cells. This pathway, thus, provides a positive feedback mechanism whereby activated T cells can promote further expansion of activated T cells. In addition, the increase of B7-1

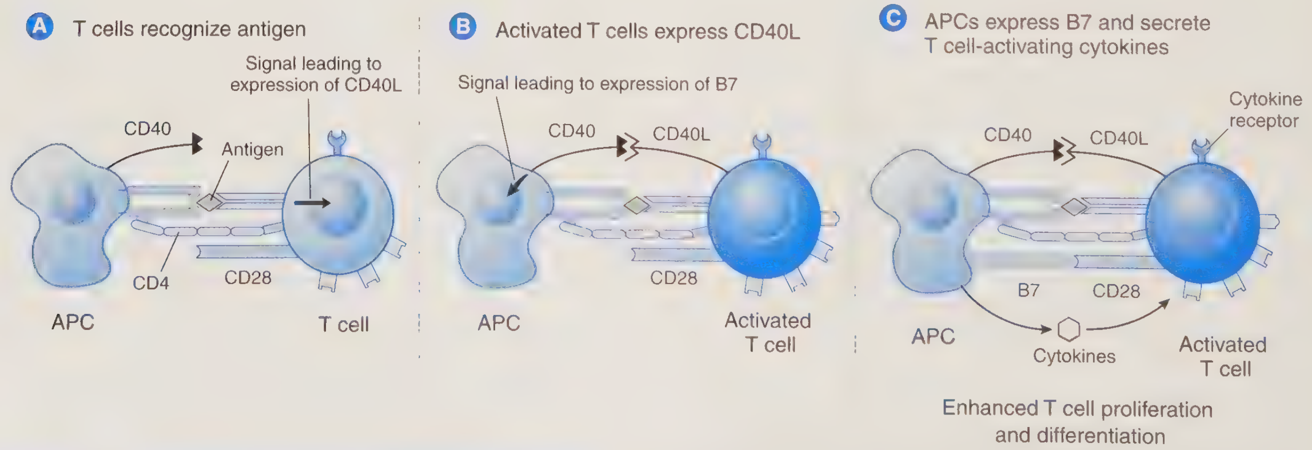

A T cells recognize antigen
Signal leading to expression of CD40L
CD40
Antigen
CD4
CD28
APC
T cell
B Activated T cells express CD40L
Signal leading to expression of B7
CD40
CD40L
CD28
APC
Activated T cell
C APCs express B7 and secrete T cell-activating cytokines
Cytokine receptor
CD40
CD40L
B7
CD28
APC
Cytokines
Activated T cell
Enhanced T cell proliferation and differentiation

Figure 43-10. Costimulation and the CD40:CD40L Interaction. **A.** An antigen-presenting cell (APC) presents MHC class II-bound antigen to a CD4$^+$ T cell. T cell recognition of antigen initiates an intracellular signaling cascade that leads to expression of CD40 ligand (CD40L) at the T cell surface. **B.** CD40L on the activated T cell binds to CD40 on the surface of the APC. Activation of CD40 generates an intracellular signaling cascade that leads to expression of B7 on the APC surface. **C.** Enhanced T cell proliferation and differentiation are promoted by costimulation of the T cell by MHC class II-antigen (which binds to the T cell receptor), CD40 (which binds to T cell CD40L), and B7 (which binds to T cell CD28). Cytokines secreted by the activated APC augment T cell proliferation and differentiation.

**TABLE 43-4** Utility of Immunosuppressive Drugs

TRANSPLANTATION

| FRONT-LINE AGENTS | SECOND-LINE AGENTS | EXPERIMENTAL AGENTS |
|---|---|---|
| Cyclosporine | Sirolimus | Leflunomide |
| Azathioprine | | Costimulation blockade |
| Glucocorticoids | ATG and OKT3 | |
| Mycophenolate mofetil | Anti-CD25 | |

AUTOIMMUNE DISEASES

| FRONT-LINE AGENTS | SECOND-LINE AGENTS | EXPERIMENTAL AGENTS |
|---|---|---|
| Methotrexate | Infliximab | Costimulation blockade |
| Glucocorticoids | Etanercept | |
| Azathioprine | Adalimumab | |
| | Leflunomide | |
| | Cyclophosphamide | |

and B7-2 molecules on macrophages is important for promoting $CD8^+$ Tc cell activation.

Because the CD40:CD40L interaction promotes numerous costimulation pathways, it has been hypothesized that blockade of CD40L could also produce tolerance. Preliminary studies have recently demonstrated that blockade of CD40L with anti-CD40L antibody can produce tolerance and long-term graft survival in animal models of organ transplantation.

## BLOCKADE OF CELL ADHESION

Except for autoimmune diseases that are purely humoral, such as myasthenia gravis, the recruitment and accumulation of inflammatory cells at sites of inflammation is an essential element of most autoimmune illnesses. Several therapies that block the migration of leukocytes from the vascular space are under investigation and show promise in inflammatory bowel disease, psoriasis, multiple sclerosis, and organ transplantation. **Efalizumab,** an inhibitor of αL/β2 integrin, was recently approved for the treatment of psoriasis. These drugs inhibit antigen presentation and cellular cytotoxicity in addition to cell migration, thus providing multiple mechanisms of action. These drugs may be predicted to increase susceptibility to infection, and further study is needed to determine whether they will become approved therapeutics.

## *Conclusion and Future Directions*

Pharmacologic suppression of adaptive immunity currently employs various approaches, ranging from the relatively low specificity of glucocorticoids and cytotoxic agents to the more specific cell signaling inhibitors and antibody therapies. Glucocorticoids induce profound suppression of the inflammatory response and immune system, but cause many adverse effects. Cytotoxic agents target replication of DNA, to which immune cells (and unfortunately some other cells, such as those in the GI epithelium) are highly susceptible. The cytotoxic agent mycophenolate mofetil is highly specific, both because lymphocytes depend on de novo purine synthesis, and because mycophenolic acid preferentially targets the inosine monophosphate dehydrogenase isoenzyme present in lymphocytes. Lymphocyte signaling inhibitors—such as cyclosporine, sirolimus and tacrolimus, which target the signal transduction pathways necessary for T cell activation—are also reasonably specific. The concept of preventing immune cell activation has been extended by experimental blockade of costimulation, especially the CD28:B7 and CD40:CD40L interactions between T cells and APCs. In organ transplantation, it may be beneficial to deplete the immune system of thymocytes specifically: antithymocyte globulin, OKT3 and daclizumab are antibodies against T-cell specific epitopes. In psoriasis, specific depletion of $CD2^{hi}$ T cells has been achieved with the recently approved drug **alefacept,** an LFA-3/Fc fusion protein. Finally, specific cytokines released by cells can sometimes be targeted. Etanercept, infliximab, and adalimumab, all of which block TNF-α activity, are examples of this rapidly expanding class of agents.

With all of these agents, it is important to recognize which have been proved effective, and which drugs are new or experimental. Table 43-4 summarizes the drugs discussed in this chapter. When treating patients, clinicians start with the front line agents, and as toxicity or ineffectiveness dictates, switch to second line drugs.

## *Suggested Readings*

Allison A. Immunosuppressive drugs: the first 50 years and a glance forward. Immunopharmacology 2000;47:63–83. (*General review of immunosuppressive drugs.*)

Allison A, Eugui E. Mycophenolate mofetil and it mechanisms of action. Immunopharmacology 2000;47:85–118. (*Review of nucleotide synthesis.*)

Janeway CA, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th Ed. New York: Garland Publishing, 2001. (*Discussion of autoimmunity and transplantation immunity.*)

Marks AR. Sirolimus for the prevention of in-stent-restenosis in a coronary artery. N Engl J Med 2003;349:1307–1309. (*Overview of recent advances in drug-coated stents.*)

Mikuls TR, Weaver AL. Lessons learned in the use of tumor necrosis factor-alpha inhibitors in the treatment of rheumatoid arthritis. Curr Rheumatol Rep 2003;5:270–277. (*Review on cytokine therapy in rheumatoid arthritis.*)

Stryer L. Biosynthesis of nucleotides. In: Biochemistry. 4th Ed. New York: W. H. Freeman and Company, 1995. (*Review of nucleotide synthesis.*)

## Drug Summary Table Pharmacology of Immunosuppression

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **INHIBITORS OF GENE EXPRESSION**<br>*Mechanism—Inhibit expression of numerous pro-inflammatory gene products* | | | | |
| **Glucocorticoids** | Transplantation<br>Autoimmune diseases | Hyperglycemia, diabetes, osteoporosis | Never stop abruptly | See Chapter 26 for a more complete discussion |
| **CYTOTOXIC AGENTS**<br>*Mechanism—Inhibit essential cellular processes* | | | | |
| **Azathioprine (AZA)** | Transplantation | AZA, MTX, MMF, and leflunomide can cause:<br>Immunosuppression<br>GI toxicity<br>Hematopoietic toxicity | | See Chapter 31 for a more complete discussion of AZA and MTX |
| **Methotrexate (MTX)** | Transplantation<br>Antineoplasia<br>Rheumatoid arthritis<br>Asthma<br>Psoriasis | | | |
| **Mycophenolic acid (MMF)** | Transplantation<br>Rheumatoid arthritis | | | |
| **Leflunomide** | Rheumatoid arthritis<br>Wegener's granulomatosis<br>Systemic lupus erythematosus<br>Myasthenia gravis | | | Significant enterohepatic recirculation |
| **Cyclophosphamide** | Antineoplasia<br>Systemic lupus erythematosus | Immunosuppression<br>Cardiotoxicity<br>Alopecia<br>Secondary cancers | | See Chapter 32 for a more complete discussion |
| **SPECIFIC LYMPHOCYTE SIGNALING INHIBITORS**<br>*Mechanism—Inhibition of IL-2 signaling and T cell activation* | | | | |
| **Cyclosporine (CsA)** | Transplantation | Nephrotoxicity<br>Neurotoxicity<br>Hepatotoxicity | | Inhibits increase in IL-2 production by activated T cells |
| **Tacrolimus (FK506)** | Transplantation<br>Atopic eczema | Nephrotoxicity | | Inhibits increase in IL-2 production by activated T cells |
| **Sirolimus (rapamycin)** | Prevention of stent restenosis<br>Transplantation | Hyperlipidemia<br>Leukopenia<br>Thrombocytopenia | | Inhibits intracellular response to IL-2<br>Major effect on stent restenosis is due to inhibition of cell cycle progression |

| | | | | |
|---|---|---|---|---|
| **CYTOKINE INHIBITION**<br>*Mechanism—Inhibit pro-inflammatory cytokines; see Notes* | | | | |
| **Etanercept** | Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis | Immunosuppression<br>Demyelinating disease | Ongoing infections | Soluble TNF-receptor dimer |
| **Infliximab** | Rheumatoid arthritis<br>Crohn's disease | Immunosuppression<br>Demyelinating disease | Ongoing infections | Monoclonal anti–TNF-α antibody; chimeric mouse-human |
| **Adalimumab** | Rheumatoid arthritis | Immunosuppression<br>Demyelinating disease | Ongoing infections | Fully human anti–TNF-α antibody |
| **Anakinra** | Rheumatoid arthritis | | Unknown | Recombinant IL-1 receptor antagonist |
| **ANTIBODIES**<br>*Mechanism—Bind to antigens on immune cells; see Notes* | | | | |
| **Anti-thymocyte globulin** | Acute transplant rejection | Profound immunosuppression<br>Cytokine release syndrome | Known hypersensitivity | Polyclonal mouse anti-human T-cell antibody |
| **OKT3** | Acute transplant rejection | Pulmonary edema<br>Cytokine release syndrome | Known hypersensitivity | Monoclonal mouse anti-human CD3 antibody |
| **Daclizumab**<br>**Basiliximab** | Transplantation<br>GVHD | Development of antibodies | Known hypersensitivity | Anti-human IL-2 receptor antibody |
| **Alemtuzumab (Campath-1)** | Chronic lymphocytic leukemia | Prolonged immunosuppression | | Humanized mouse anti-human CD52 antibody |
| **INHIBITION OF COSTIMULATION**<br>*Mechanism—Inhibit activation of immune effector cells* | | | | |
| **CTLA-4 Ig** | Investigational | Unknown | Unknown | CD40 fused with IgG; binds to B7 on antigen presenting cells |
| **Anti-CD40L** | Investigational | Unknown | Unknown | Antibody that binds to CD40L on antigen presenting cells |
| **BLOCKADE OF CELL ADHESION**<br>*Mechanism—Inhibit immune cells from migrating to site of inflammation* | | | | |
| **Efalizumab** | Psoriasis | Unknown | Unknown | Inhibits αL/β2 integrin |
| **Natalizumab** | Investigational | Unknown | Unknown | In clinical trials for multiple sclerosis and Crohn's disease; inhibits α4 integrin |

# 44

# Integrative Inflammation Pharmacology: Peptic Ulcer Disease

Dalia S. Nagel and Helen M. Shields

**Introduction**
**Case**
- First Episode
- Second Episode

**Physiology of Gastric Acid Secretion**
- Neurohormonal Control of Gastric Acid Secretion
- Phases of Gastric Acid Secretion
- Protective Factors

**Pathophysiology of Peptic Ulcer Disease**
- Gastroduodenal Mucosal Defenses
- Helicobacter pylori
- NSAIDs
- Acid Hypersecretion
- Other Factors

**Pharmacologic Classes and Agents**
- Agents That Decrease Acid Secretion
    - *H2 Receptor Antagonists*
    - *Proton Pump Inhibitors*
    - *H2 Receptor Antagonists versus Proton Pump Inhibitors*
    - *Anticholinergic Agents*
- Agents That Neutralize Acid
- Agents That Promote Mucosal Defense
    - *Coating Agents*
    - *Prostaglandins*
- Agents That Modify Risk Factors
    - *Diet, Tobacco, and Alcohol*
    - *NSAIDs*
    - *Treatment of H. pylori Infection*

**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

A peptic ulcer is a break in the mucosa of the stomach (gastric ulcer) or duodenum (duodenal ulcer). Four-and-a-half million people in the United States suffer from active peptic ulcer disease, and five-hundred thousand new cases of peptic ulcer disease are diagnosed each year. The lifetime prevalence of peptic ulcer disease is approximately 10%. Furthermore, the estimated annual cost for treatment exceeds one billion dollars.

Various pathophysiologic mechanisms of peptic ulcer disease, and thus clinical management requires multiple pharmacologic agents. This chapter describes the physiology of gastric acid secretion and the pathophysiology underlying the formation of peptic ulcers. The corresponding pharmacologic agents used in the treatment of peptic ulcer disease are then discussed in relation to the pathophysiology that is interrupted by these drugs.

## ■ Case

### FIRST EPISODE

Tom is a 24-year-old graduate student in good health. He smokes approximately two packs of cigarettes a day, and drinks five cups of coffee a day. He is currently under stress because of the impending deadline for his computer science thesis. In addition, he has been taking two aspirin daily for the past 2 months because of a knee injury he sustained while skiing during winter vacation.

For the past 2 weeks, he has noted a burning pain in his upper abdomen occurring 1 to 2 hours after eating. In addition, the pain frequently wakens him at approximately 3 AM. His pain is usually relieved by eating and by taking over-the-counter antacids (TUMS®).

When the pain increases in intensity, Tom decides to visit his internist, Dr. Smith, at University Health Services.

Dr. Smith notes that the abdominal examination is normal except for epigastric tenderness. Dr. Smith discusses diagnostic options with Tom, including an upper gastrointestinal series and an endoscopic exam. Tom chooses to undergo an endoscopic examination. During the endoscopic examination, an ulcer is identified in the proximal portion of the duodenum on the posterior wall. The ulcer is 0.5 cm in diameter. In addition, a mucosal biopsy of the gastric antrum is performed for detection of *Helicobacter pylori.*

Tom is diagnosed with a duodenal ulcer. Dr. Smith prescribes omeprazole, a proton pump inhibitor. The following day, when the pathology report indicates the presence of an *H. pylori* infection, Dr. Smith prescribes bismuth, clarithromycin, and amoxicillin in addition to the proton pump inhibitor. Dr. Smith also advises Tom to stop smoking and drinking coffee and, importantly, to avoid taking aspirin.

## SECOND EPISODE

Tom has no medical problems until 10 years later, when he develops carpal tunnel syndrome. He begins to take several aspirin daily for the pain. One month later, Tom develops a burning pain in his upper abdomen. After vomiting "coffee grounds" material and noticing that his bowel movements appear black, he decides to visit his internist. By endoscopy, the doctor diagnoses Tom with a gastric ulcer that has caused an acute gastrointestinal hemorrhage. The doctor explains to Tom that he has had a recurrence of his peptic ulcer disease. His breath test is negative for *H. pylori,* and he is treated with ranitidine, an H2 receptor antagonist, and antacids. Tom is told to stop taking aspirin, which is the probable cause of this recurrence. The doctor goes over with Tom which pain-relieving medications are considered nonsteroidal anti-inflammatory drugs (NSAIDs).

Two weeks pass, and Tom informs Dr. Smith that the pain in his wrist has become unbearable and that he must continue taking aspirin to be able to concentrate at work. Dr. Smith tells him that he can take aspirin as long as he switches his antiulcer medication from an H2 antagonist to a proton pump inhibitor while he is taking the NSAID.

## QUESTIONS

- 1. What risk factors did Tom have for the development of peptic ulcer disease? What are the roles of *H. pylori* and NSAIDs in this disease?
- 2. Why was Tom given a proton pump inhibitor for his first episode of peptic ulcers? Why was an H2 antagonist prescribed for his second episode, and then a proton pump inhibitor when he insisted on using aspirin as an analgesic?
- 3. Concerning treatment for *H. pylori,* why was Tom given clarithromycin as opposed to metronidazole?

# PHYSIOLOGY OF GASTRIC ACID SECRETION

## NEUROHORMONAL CONTROL OF GASTRIC ACID SECRETION

The **parietal cells,** located in the oxyntic glands of the fundus and body of the stomach, secrete hydrochloric acid and intrinsic factor. The parietal cell actively transports $H^+$ across the apical canalicular membranes via $H^+/K^+$ ATPases (proton pumps), extruding intracellular $H^+$ in exchange for extracellular $K^+$. Three neurohormonal secretagogues (stimulants of secretion that bind to specific receptors on the basolateral membrane of the secretory cell) regulate this process: **histamine, gastrin,** and **acetylcholine** (ACh). Each of these secretogogues activates receptors and initiates the biochemical changes necessary for active transport of $H^+$ out of the parietal cell.

Histamine, released by **enterochromaffin-like (ECL) cells** (located in and adjacent to the oxyntic glands) and by mast cells in the lamina propria, binds to the histamine **H2 receptor** on the parietal cell basolateral membrane, which increases intracellular cyclic adenosine monophosphate (cAMP) by activation of adenylyl cyclase. The increase in cAMP activates cAMP-dependent protein kinase, which phosphorylates a membrane-bound enzyme, the apical $H^+/K^+$ ATPase. Phosphorylation of this exchanger activates extrusion of $H^+$ from the parietal cell (Fig. 44-1).

Gastrin is secreted into the blood stream by **G cells** in the gastric antrum, whereas acetylcholine (Ach) is released from postganglionic nerves with cell bodies located in the submucosa (Meissner's plexus). These substances bind to their respective receptors on the parietal cell, which increase cytoplasmic accumulation of calcium ($Ca^{2+}$). $Ca^{2+}$ binds to calmodulin and stimulates adenylyl cyclase. $Ca^{2+}$ also activates protein kinase C, which phosphorylates and activates $H^+/K^+$ ATPase to increase secretion of $H^+$ into the gastric lumen (Fig. 44-1).

While histamine, gastrin and ACh increase parietal acid secretion, **somatostatin-secreting D cells** and **prostaglandins** limit the extent of gastric acid secretion. Somatostatin decreases acid secretion via three mechanisms. Somatostatin: (1) inhibits gastrin release from G cells by a paracrine mechanism; (2) inhibits histamine release from ECL cells and mast cells; and (3) directly inhibits parietal cell secretion. Prostaglandins reduce basal and stimulated gastric acid secretion, and enhance epithelial cell bicarbonate secretion, mucus production, cell turnover, and local blood flow, thereby enhancing mucosal resistance to tissue injury.

## PHASES OF GASTRIC ACID SECRETION

Gastric secretions increase considerably during a meal. There are three phases of gastric acid secretion.

The **cephalic phase** includes responses to sight, taste, smell, and thought of food. "Sham feedings," experiments in which food is chewed but not swallowed, trigger a rise in acid secretion mediated by vagal stimulation and increased gastrin secretion.

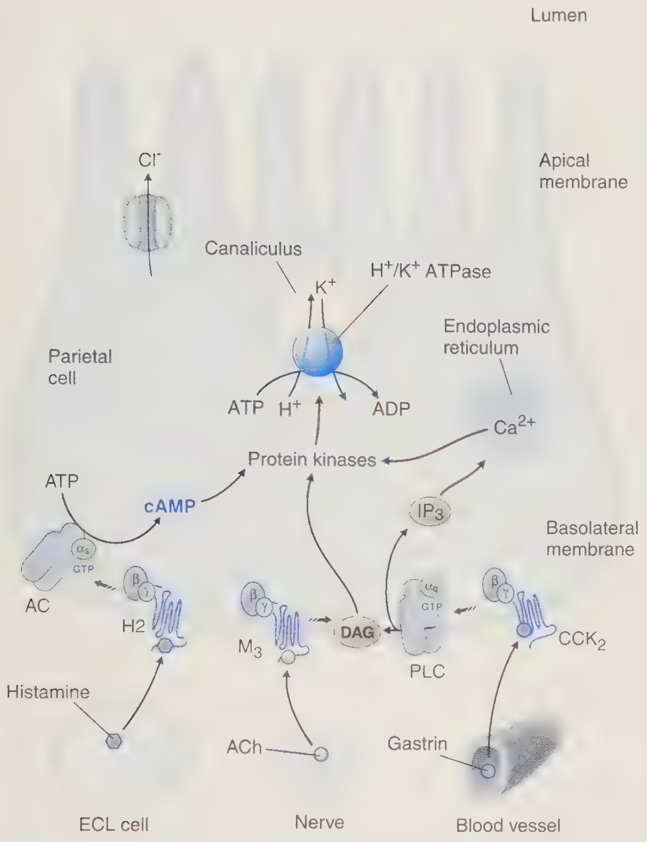


**Figure 44-1. Control of Parietal Cell Acid Secretion.** Stimulation of parietal cell acid secretion is modulated by paracrine (H2, histamine), neuroendocrine (ACh, acetylcholine), and endocrine (gastrin) pathways, which activate their respective receptors (H2, $M_3$, and $CCK_2$). H2 receptor activation increases cAMP, which activates protein kinases. $M_3$ and $CCK_2$ receptor activation stimulate release of $Ca^{2+}$ by the $G_q$-mediated $IP_3$/DAG pathway; these signals also stimulate protein kinase activity. Protein kinase activation results in phosphorylation and activation of the canalicular membrane $H^+/K^+$ ATPase, which pumps $H^+$ ions into the stomach lumen. An apical membrane $Cl^-$ channel couples $Cl^-$ efflux to $H^+$ efflux, and an apical membrane $K^+$ channel (not shown) recycles $K^+$ out of the cell. The net result of this process is the rapid extrusion of HCl into the stomach lumen.

Mechanical distension of the stomach and the ingestion of amino acids and peptides stimulate the **gastric phase.** Distension activates stretch receptors in the wall of the stomach that are linked to short intramural nerves and vagal fibers. Luminal nutrients, such as amino acids, are strong stimulants for gastrin release. In addition to stimulating acid secretion, directly gastrin travels via the blood to the oxyntic mucosa and stimulates ECL cells to release histamine. An important negative feedback of acid secretion in this phase is the inhibition of the release of gastrin from the antral G cells by the presence of acid (pH < 3). Acid secretion is also inhibited by antral D cell release of somatostatin.

The **intestinal phase** involves the stimulation of gastric acid secretion by digested protein in the intestine. Gastrin plays a large role in mediating this phase as well.

## PROTECTIVE FACTORS

The factors that act to protect the gastric mucosa are gastric mucus, gastric and duodenal bicarbonate, prostaglandins (discussed above, and in Chapter 40), restitution (repair), and blood flow. The epithelial cells of the stomach secrete mucus, which acts as a lubricant that protects the mucosal cells from abrasions. **Mucus** is composed of glycoproteins and is hydrophilic, with viscous and gel-forming properties. This allows for an uninterrupted layer of water at the luminal surface of the epithelium, thus restricting the acidic environment to the lumen of the stomach. Mucus secretion is inhibited by NSAIDs and anticholinergic medications; whereas prostaglandins stimulate mucus production. In addition, *Helicobacter pylori* disturbs the mucus layer.

Like mucus, the major function of **bicarbonate** is to protect the gastric epithelium by neutralizing acid at the gastric mucosal surface. Bicarbonate is secreted by epithelial cells at the surface of the gastric lumen and in gastric pits. Bicarbonate secretion in the duodenum occurs at the surface of duodenal mucosal epithelial cells and where it serves to neutralize the acid entering the intestine from the stomach.

The mucosa has **restitutive** properties as well. Epithelial cells of the gastric lumen are able to repair damage to the mucosa quickly. Damage is repaired through the process of migration by undamaged epithelial cells that migrate along the basement membrane to fill in defects created by the sloughing of injured cells.

The final protective factor of the mucosa is **blood flow.** Blood flow to the area of the mucosa carries away acid that has diffused across a damaged mucus layer.

# PATHOPHYSIOLOGY OF PEPTIC ULCER DISEASE

## GASTRODUODENAL MUCOSAL DEFENSES

A peptic ulcer is a break in the lining of the stomach or duodenum involving the mucosa, muscularis mucosa, submucosa and, in some cases, the deeper layers of the muscle wall. This compromise of mucosal integrity can cause pain, perforation, bleeding, obstruction, and even death. Peptic ulcers are caused by an imbalance between the protective factors and damaging factors in the gastrointestinal mucosa (Table 44-1). This section describes the main pathophysiologic mechanisms involved in the formation of ulcers, the two most common causes being *Helicobacter pylori* infection and NSAID use.

## HELICOBACTER PYLORI

***Helicobacter pylori*** is a Gram-negative, spiral-shaped bacterium. It is the most common cause of non-NSAID associated peptic ulcer disease. *H. pylori* has been found in the antrum of a significant number of patients with duodenal ulcers and gastric ulcers. When Tom first presented to Dr. Smith in the introductory case, *H. pylori* was found. Eradication of *H. pylori* in patients with ulcers leads to a lower recurrence or relapse rate.

**TABLE 44-1** Factors That Protect and Damage Gastric Mucosa

| PROTECTIVE FACTORS | DAMAGING FACTORS |
|---|---|
| Mucus | Acid secretion |
| Bicarbonate | Pepsin |
| Prostaglandins | NSAIDs |
| Blood flow | *H. pylori* |
| Restitution (repair) | Smoking |
| | Ischemia |
| | Bile acids |
| | Alcohol |

This finding, along with the fact that many ulcer patients are infected with *H. pylori,* constitutes the major evidence for a causal role for *H. pylori* in peptic ulcer disease.

*H. pylori* is able to live in the acidic environment of the stomach. The initial infection involves ingestion of the microaerophilic bacterium, which uses its 4–6 flagellae and spiral shape to move in corkscrew fashion through the gastric mucous layer. *H. pylori* attaches to the surface of the gastric epithelial cells via adhesion molecules. In the duodenum, *H. pylori* attaches only to areas that contain gastric epithelial cells which arise as a result of excess acid damage to the duodenal mucosa (gastric metaplasia). *H. pylori* is able to live in such a hostile environment partly because of its production of the enzyme **urease.** This enzyme converts urea to ammonia. The ammonia buffers the $H^+$ and forms ammonium hydroxide, creating an alkaline cloud around the bacterium, protecting it from the acidic environment of the stomach.

*H. pylori*'s virulence factors cause damage to the host. Urease is one of these damaging factors because it is an antigen that causes a strong immune response. In addition, the ammonium hydroxide produced by urease causes gastric epithelial cell injury. Other virulence factors include lipopolysaccharides (endotoxins), which are part of the bacteria's outer membrane, as well as a lipase and a protease, which are secreted by the bacteria and degrade the gastric mucosa. Cytotoxicity caused by *H. pylori* has also been linked to two proteins associated with vacuolating cytotoxins, cag A and vac A.

The persistence of *H. pylori* can be traced, in part, to the inappropriate immune responses it elicits. Instead of the normal $T_H2$ mucosal immunity response, which controls luminal infection with an antibody response (IgA), the *H. pylori* organism elicits a $T_H1$ response. This $T_H1$ response elicits cytokine production that induces inflammation and the formation of autoantibodies, leading to epithelial cell damage.

Several additional mechanisms characterize for *H. pylori*-induced peptic ulcer disease (Fig. 44-2). Acid secretion is increased in patients with *H. pylori*-associated duodenal ulcers. This is thought to result from increased levels of circulating gastrin, causing increased parietal cell replication and increased acid output. Gastrin secretion is elevated because of the ammonia generated by *H. pylori,* producing an alkaline environment near the G cells and, thereby, stimulating gastrin release. In addition, antral somatostatin concentration and the number of antral somatostatin-producing D cells are lower in *H. pylor*-infected patients, resulting in increased gastrin release. *H. pylori* also causes decreased duodenal bicarbonate secretion, which weakens the protective mechanisms of the duodenal mucosa.

The presence of *H. pylori* infection can be detected using the **$^{13}C$ urea breath test,** which is based on the organism's production of urea, whereby $^{13}CO_2$ is released urease. In this test, $^{13}CO_2$ is formed from ingested $^{13}C$ -urea if *H. pylori,* with its urease enzyme, is present in the stomach. This is the current best diagnostic test to determine the presence of the organism. Other methods of detecting *H. pylori* include histologic examination of a gastric mucosal biopsy, as was performed in Tom's case, as well as serologic testing for antibodies and a stool antigen test.

## NSAIDS

More than 100,000 patients are hospitalized each year for NSAID-associated gastrointestinal complications, and gas-

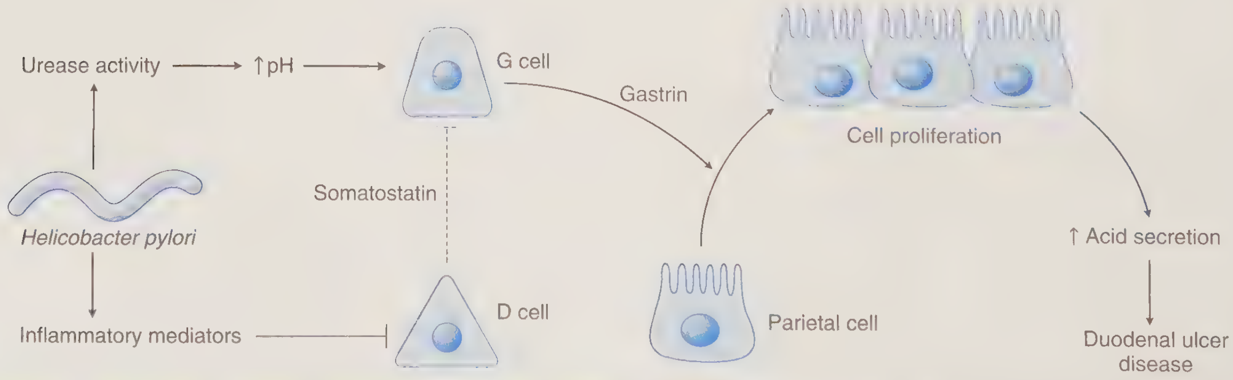


**Figure 44-2. The Role of *H. Pylori* in Duodenal Peptic Ulcer Disease.** Two of the mechanisms by which *H. pylori* infection predisposes to peptic ulcer disease are illustrated. First, the inflammatory mediators elicited by *H. pylori* inhibit somatostatin secretion by D cells in the antrum of the stomach. Decreased D cell somatostatin secretion causes disinhibition of gastrin release from G cells. Second, the ammonium hydroxide produced by *H. pylori*-derived urease increases gastric pH, which, in turn, stimulates gastrin secretion. Activation of gastrin release by both of these mechanisms leads to parietal cell proliferation, which increases the functional capacity of the gastric mucosa to secrete $H^+$ ions and, thereby, predisposes to the development of duodenal ulcer disease.

trointestinal bleeding has a 5–10% mortality rate in these patients. The gastrointestinal tract is the most common target for the adverse effects of NSAID use.

NSAID-associated gastrointestinal damage is attributable to both *topical injury* as well as the *systemic effects* of the NSAID (Fig. 44-3). Most NSAIDs are weak organic acids. In the acidic environment of the stomach, these drugs are neutral compounds that can cross into the gastric epithelial cells. Once they enter epithelial cells, NSAIDs are in a neutral pH environment. In the neutral environment, they are re-ionized and trapped inside the cell where they cause cell damage. This mechanism constitutes the local gastrointestinal injury associated with NSAID use.

NSAIDs also cause systemic injury to the gastrointestinal wall lining, largely because of decreased mucosal prostaglandin synthesis. The two cyclooxygenase enzymes, the prostaglandin H synthases, are responsible for catalyzing formation of prostaglandins from arachidonic acid (see Chapter 40, Pharmacology of Eicosanoids). Cyclooxygenase-1 (COX-1) is responsible for gastrointestinal mucosal integrity, while cyclooxygenase-2 (COX-2) is responsible for inflammatory processes. Nonselective NSAIDs can inhibit both of these enzymes, leading to gastrointestinal damage.

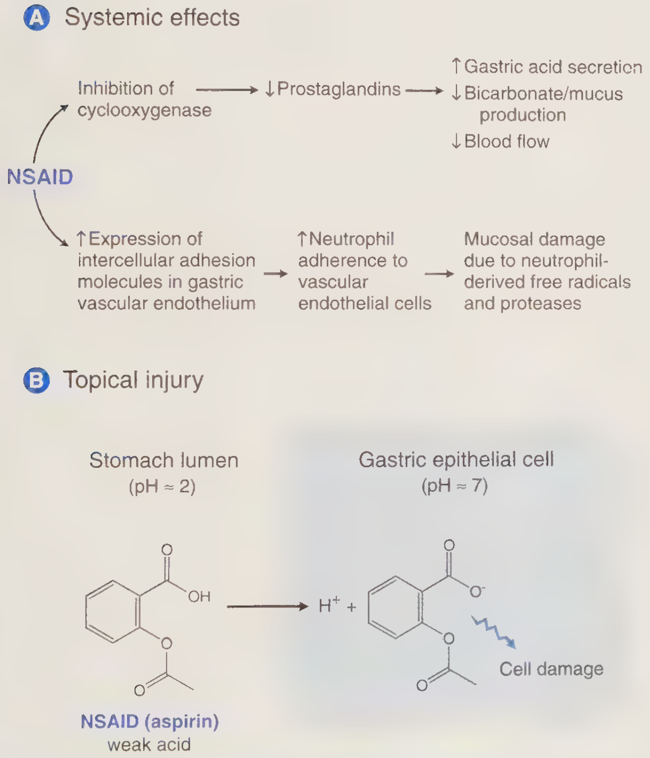

A Systemic effects
↑Gastric acid secretion
Inhibition of cyclooxygenase
↓Prostaglandins
↓Bicarbonate/mucus production
↓Blood flow
NSAID
↑Expression of intercellular adhesion molecules in gastric vascular endothelium
↑Neutrophil adherence to vascular endothelial cells
Mucosal damage due to neutrophil-derived free radicals and proteases
B Topical injury
Stomach lumen (pH ≈ 2)
Gastric epithelial cell (pH ≈ 7)
OH
O
H⁺ +
O⁻
Cell damage
NSAID (aspirin)
weak acid

**Figure 44-3. Role of NSAIDs in Peptic Ulcer Disease.** NSAID-associated peptic ulcer disease is a result of both systemic effects and topical injury. **A.** Systemic effects: NSAIDs inhibit cyclooxygenase and, thereby, decrease the production of prostaglandins. Because prostaglandins activate $G_i$ and, thereby, decrease the generation of cAMP in gastric parietal cells, decreased prostaglandin production causes increased gastric acid secretion. Decreased prostaglandins also decrease bicarbonate production, mucus production, and blood flow in the stomach. An additional systemic effect involves the increased expression of intercellular adhesion molecules (ICAMs) in the vascular endothelium of the stomach, which increases neutrophil adherence to the vascular endothelial cells. Neutrophils release free radicals and proteases that cause mucosal damage. **B.** Topical effects: NSAIDs induce local injury via ion trapping. From the lumen of the stomach, the drug enters the gastric epithelial cell in its protonated (uncharged) form. In the neutral environment of the cytoplasm, the NSAID is ionized and trapped inside the cell, causing cell damage.

NSAIDs, such as aspirin, are used as analgesic, antipyretic, and anti-inflammatory agents. Their anti-inflammatory properties can be explained in large part by their inhibition of cyclooxygenase-2. In contrast, the inhibition by NSAIDs of cyclooxygenase-1 leads to gastroduodenal ulceration, because inhibition of prostaglandin synthesis removes one of the protective mechanisms that maintains the integrity of the gastric mucosa.

New drugs, such as celecoxib and rofecoxib, are selective cyclooxygenase-2 inhibitors that do not inhibit prostaglandin synthesis in the gastrointestinal mucosa. These agents, at present, seem to cause fewer ulcers and gastrointestinal bleeds than do conventional NSAIDs (see below).

Although there is much evidence for NSAID damage caused by inhibition of prostaglandin synthesis, there are other systemic mechanisms by which NSAIDs may cause ulcers. For example, NSAIDs increase expression of intercellular adhesion molecules in the gastric vascular endothelium. Increased adherence of neutrophils to the gastric vascular endothelium causes release of free radicals and proteases that can lead to mucosal damage.

## ACID HYPERSECRETION

Acid hypersecretion is another important causative factor in peptic ulcer disease. **Zollinger–Ellison syndrome** and **Cushing's ulcers** are two clinical examples in which hyperacidity leads to peptic ulcer disease. In Zollinger–Ellison syndrome, a gastrin-secreting tumor of the non-beta endocrine cells of the pancreas leads to increased acid secretion. In Cushing's ulcers, seen in central nervous system disasters, heightened vagal tone causes gastric hyperacidity. The local hyperacidic environment induced by these conditions can result in peptic ulcer disease.

## OTHER FACTORS

Pepsin is a digestive enzyme secreted from gastric chief cells as the inactive precursor pepsinogen. Studies have suggested a role for pepsin in ulcer formation. Cigarette smoking is associated with peptic ulcer disease because of its impairment of mucosal blood flow and healing and its decreasing of pancreatic bicarbonate production. Caffeine ingestion (increased acid secretion), alcoholic cirrhosis, corticosteroid use, and genetic influences are associated with peptic ulcer disease as well. In addition, chronic psychological stress may occasionally be an important cause of peptic ulcer disease. In the introductory case, Tom was under severe stress to finish his computer science thesis, smoked cigarettes, and drank a lot of coffee. These factors may have contributed to his development of an ulcer.

## PHARMACOLOGIC CLASSES AND AGENTS

Because several pathophysiologic mechanisms can lead to peptic ulcer disease, clinical management requires consideration of multiple pharmacologic options. The available agents can be divided into drugs that: (1) decrease acid secretion; (2) neutralize acid; (3) promote mucosal defense; and (4) modify risk factors.

## AGENTS THAT DECREASE ACID SECRETION

### H2 Receptor Antagonists

The discovery of **H2 receptor antagonists** by Black and colleagues in the 1970s changed the treatment of peptic ulcer disease significantly. These investigators identified a second histamine receptor and elucidated its role in gastric acid secretion. H2 receptor antagonists reversibly and competitively inhibit the binding of histamine to H2 receptors, resulting in suppression of gastric acid secretion. H2 receptor antagonists also decrease gastrin- and acetylcholine-induced gastric acid secretion.

Four H2 receptor antagonists are available: **cimetidine, ranitidine, famotidine,** and **nizatidine** (Fig. 44-4). H2 receptor antagonists are absorbed rapidly from the small intestine. Peak plasma concentration is reached within 1–3 hours. Elimination of H2 receptor antagonists involves both renal excretion and hepatic metabolism. Therefore, it is important that the dosage of these drugs be decreased for patients with liver or kidney failure. (An exception is nizatidine, which is eliminated primarily by the kidney.)

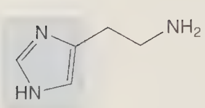
All four drugs are well tolerated in general, with occasional minor side effects, including diarrhea, headache, muscle pain, constipation, and fatigue. H2 receptor antagonists may induce confusion and hallucinations in some patients. These CNS side effects, however, are uncommon and are usually associated with intravenous administration of the H2 receptor antagonist. Additional side effects specific to cimetidine are discussed below.

Several clinically significant drug–drug interactions may occur with H2 receptor antagonists. For example, ketoconazole, a drug that requires an acidic medium for gastric absorption, will have reduced uptake in the more alkaline environment that H2 receptor antagonists create. In addition, H2 receptor antagonists compete for renal tubular secretion of creatinine and certain medications, such as procainamide.

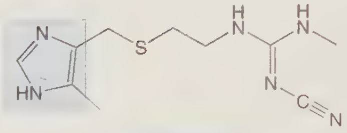
Cimetidine inhibits cytochrome P450 and, thus, can interfere with hepatic metabolism of other drugs. These drug–drug interactions are usually not clinically significant, except for drugs that have a narrow therapeutic index. For example, the interactions of cimetidine with lidocaine, phenytoin, quinidine, theophylline, and warfarin may result in toxic levels of these drugs, because of decreased metabolism. Cimetidine appears to inhibit cytochrome P450 to a greater extent than the other H2 receptor antagonists; other H2 receptor antagonists may, therefore, be preferred when a patient is receiving other medications.

Cimetidine, the first H2 receptor antagonist developed, is distributed throughout the body and across the placenta. Because it is secreted into breast milk, cimetidine is not recommended for use during pregnancy or when nursing. Cimetidine can have antiandrogenic effects because it acts as an antagonist at the androgen receptor, resulting in gynecomastia (enlarged breasts) in some men and galactorrhea (discharge of milk) in women.

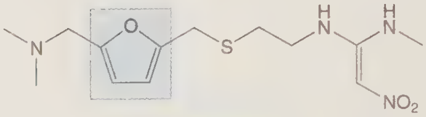
On a scale of relative potency, ranitidine is more potent than cimetidine; however, famotidine is both more potent and has a longer half-life than either cimetidine or ranitidine.



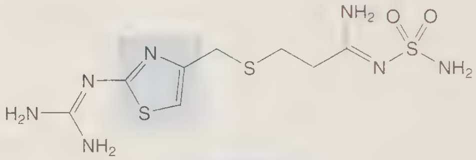
Histamine
(imidazole ring)



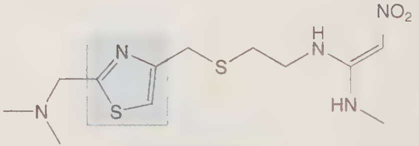
**Cimetidine**
(imidazole ring)



**Ranitidine**
(furan ring)



**Famotidine**
(thiazole ring)



**Nizatidine**
(thiazole ring)

**Figure 44-4. Histamine H2 Receptor Antagonists.** H2 receptor antagonists share structures related to histamine, providing a structural rationale for inhibition of the H2 receptor. Differences in the flexible side chain and polar hydrogen-bonding groups differentiate the H2 receptor blockers structurally. For a more detailed description of the structure of these agents, see the legend to Fig. 41-4.

### Proton Pump Inhibitors

**Proton pump inhibitors** block the $H^+/K^+$ ATPase, or proton pump. These drugs are superior to H2 receptor antagonists in the suppression of acid and in promoting healing of peptic ulcers. In the acidic environment of the parietal cell canaliculi, the proton pump inhibitor prodrug is converted to a **sulfenamide,** its active form (Fig. 44-5). The drug is enteric-

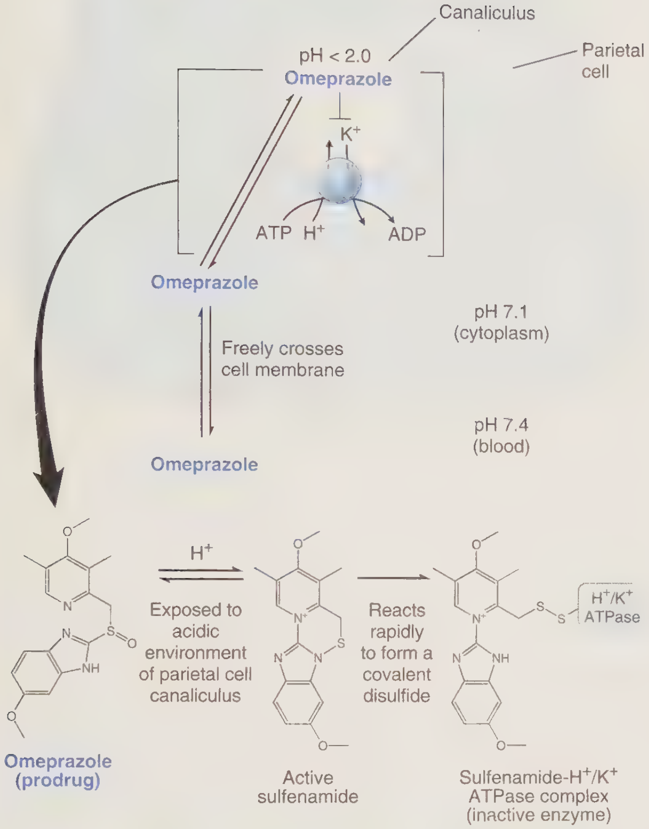

Canaliculus
Parietal cell
pH < 2.0
Omeprazole
K+
ATP H+
ADP
Omeprazole
pH 7.1 (cytoplasm)
Freely crosses cell membrane
pH 7.4 (blood)
Omeprazole
H+
Exposed to acidic environment of parietal cell canaliculus
Reacts rapidly to form a covalent disulfide
H+/K+ ATPase
Omeprazole (prodrug)
Active sulfenamide
Sulfenamide-H+/K+ ATPase complex (inactive enzyme)

**Figure 44-5. Mechanism of Action of Omeprazole, a Proton Pump Inhibitor.** Omeprazole freely enters the cytoplasm of the parietal cell (pH 7.1) in uncharged form. In the acidic environment of the parietal cell canalicular system (pH < 2.0), omeprazole is converted to its active sulfenamide form. The sulfenamide reacts with a cysteine residue on the $H^+/K^+$ ATPase to form a covalent disulfide bond. Covalent modification of the $H^+/K^+$ ATPase inhibits the activity of the proton pump and, thereby, prevents acid secretion.

coated to prevent premature activation. The sulfenamide reacts with a cysteine residue on the $H^+/K^+$ ATPase, forming a covalent disulfide bond. This reaction inhibits the activity of the proton pump irreversibly and leads to nearly complete suppression of acid secretion. For acid secretion to resume, the parietal cell must synthesize new $H^+/K^+$ ATPase, which requires approximately 18 hours.

Proton pump inhibitors are generally well tolerated. Proton pump inhibitors, however, cause such a large decrease in acid secretion that enteric infections, such as *Salmonella,* may occur. A concern with the use of proton pump inhibitors is the large accompanying increase in plasma gastrin. The decreased acid secretion resulting from proton pump inhibitor therapy leads to increased gastrin release, because of lack of negative feedback inhibition on the G cell by gastric acid. Gastrin's trophic effect on the mucosa can result in hyperplasia of ECL cells. Although rats treated for long durations with omeprazole developed gastric carcinoid tumors, these tumors have not been observed in humans. Patients with Zollinger–Ellison syndrome usually develop ECL hyperplasia and some develop carcinoid tumors, but no increase in carcinoid tumors has been found in Zollinger–Ellison patients taking proton pump inhibitors. Hypergastrinemia can result in rebound hypersecretion of acid once the proton pump inhibitor is discontinued.

**Omeprazole** is the prototype proton pump inhibitor. The $H^+/K^+$ ATPase pump binds omeprazole irreversibly, leading to inhibition of acid secretion that can persist for many hours. The side effects of omeprazole include headache, nausea, disturbed bowel function, and abdominal pain. Omeprazole and other proton pump inhibitors are metabolized in the liver by the cytochrome P450 system; for patients with liver disease, a reduction in the dose of the proton pump inhibitor should be considered. In addition, drug–drug interactions caused by the reduced metabolism of drugs, such as diazepam and phenytoin, can result in toxic plasma levels of these drugs when taken concomitantly with omeprazole.

Several other proton pump inhibitors, such as **lansoprazole, rabeprazole, pantoprazole,** and **esomeprazole** have been developed. The different proton pump inhibitors have similar rates of absorption, bioavailability and metabolism; however, only omeprazole has been found to have interactions with other drugs metabolized by the cytochrome P450 system. Although clinically significant interactions generally do not occur, awareness should be high in patients taking omeprazole and warfarin, phenytoin, diazepam, or carbamazepine. (See Box 44-1 for discussion of genetic polymorphisms and the metabolism of proton pump inhibitors.)

## H2 Receptor Antagonists versus Proton Pump Inhibitors

Proton pump inhibitors are used to treat *H. pylori*-associated ulcers and hemorrhagic ulcers, and to allow continued use of NSAIDs in a patient with a known peptic ulcer. Proton pump inhibitors are suggested for the treatment of peptic ulcer disease when there is an accompanying *H. pylori* infection because they contribute to eradication of the infection by inhibiting the growth of *H. pylori.*

Proton pump inhibitors are effective in preventing recurrent hemorrhagic ulcers because they help maintain clot integrity. Clot formation involves processes that are susceptible to pH. An intravenous infusion of omeprazole is able to maintain the intragastric pH above 6.0, thereby supporting platelet aggregation and the stability of the clot. In addition to the prevention of ulcer bleeding, intravenous infusion of a proton pump inhibitor is particularly useful in patients with bleeding who are unable to take oral medications, and in patients with compromised gastrointestinal absorption. Intravenous pantoprazole is currently available in the United States. A single IV dose of pantoprazole (80 mg) is effective in reducing acid secretion for at least 20 hours.

Proton pump inhibitors are superior to H2 receptor antagonists (ranitidine) for healing of NSAID-associated gastric and duodenal ulcers when the patient continues use of NSAIDs. This is likely based on the differences in the ability of the two drug classes to sustain an increased gastric pH.

H2 receptor antagonists may be chosen over proton pump inhibitors in certain situations. H2 receptor antagonists have been in use longer than proton pump inhibitors. Therefore, their adverse effects are well known. This may be an important

### BOX 44-1. Metabolism of Proton Pump Inhibitors

A consideration when prescribing proton pump inhibitors is their metabolism. Omeprazole, lansoprazole, and pantoprazole are almost completely metabolized in the liver to less active or inactive metabolites, which are then rapidly cleared from the body. Their metabolism involves two cytochrome P450 isoenzymes in the liver, CYP2C19 (S-mephenytoin hydroxylase) and CYP3A4 (nifedipine hydroxylase). Studies have shown that different individuals have different rates of metabolism and clearance of these drugs because of the genetic polymorphisms of the CYP2C19 isoenzyme.

Two polymorphisms of CYP2C19, CYP2C19m1, and CYP2C19m2, alter the activity of this enzyme. These polymorphisms exist most commonly in Asian populations. Carriers of two copies of a polymorphism are considered "poor metabolizers" of proton pump inhibitor drugs. Carriers of one polymorphism are intermediate–extensive metabolizers, because their rate of CYP2C19-mediated drug metabolism is reduced, but not to the extent of people with two copies of the polymorphism.

The extent of proton pump inhibitor effects on intragastric pH and plasma gastrin is dependent on the CYP2C19 polymorphism that is present. Therefore, administration of these drugs to carriers of the CYP2C19 polymorphisms can delay clearance of the drug, producing potent suppression of acid secretion and potentially significant drug–drug interactions.

In the future, screening for the presence of CYP2C19 polymorphisms may guide the administration of proton pump inhibitors. This information will allow physicians to determine which proton pump inhibitor is most appropriate for each patient and what dosage should most effectively favor acid inhibition and while preventing drug–drug interactions.

consideration for pregnant women because H2 receptor antagonists (with the exception of cimetidine, as noted above) have proven to be safe in pregnancy. In addition, H2 receptor antagonists are less expensive than proton pump inhibitors. The possibility that proton pump inhibitors may cause gastric carcinoid tumors is sometimes raised as a concern for long-term proton pump inhibitor therapy. This association, however, has not been observed in humans.

In the introductory case, Tom was first given a proton pump inhibitor because he was found to have an associated *H. pylori* infection. For the second episode, however, he was given an H2 receptor antagonist because there was no associated *H. pylori* infection, and H2 receptor antagonists are more affordable. After realizing that Tom needed to continue using NSAIDs, however, the doctor prescribed a proton pump inhibitor to allow for the simultaneous use of NSAIDs.

### Anticholinergic Agents

Anticholinergic agents, such as **dicyclomine,** can be used in the treatment of peptic ulcer because they inhibit parietal cell muscarinic ACh receptors, thereby decreasing gastric acid secretion. However, anticholinergic agents are seldom used in the treatment of ulcers because they are not as effective as H2 receptor antagonists or proton pump inhibitors. In addition, they have many side effects, including mouth dryness, vision blurring, cardiac arrhythmias, and urinary retention.

## AGENTS THAT NEUTRALIZE ACID

**Antacids** are used on an as-needed basis for symptomatic relief of dyspepsia. They neutralize gastric hydrochloric acid by reacting with acid to form water and salt. The most widely used antacids are mixtures of **aluminum hydroxide** and **magnesium hydroxide** (Mylanta, Maalox). Magnesium and aluminum react with $HCO_3^-$ from pancreatic secretions and with phosphates from the diet to yield salts. The hydroxide and $H^+$ ions react to form water. Patients with renal failure should avoid magnesium-containing antacids because they can lead to hypermagnesemia. In addition, antacids that contain aluminum can bind phosphate, resulting in hypophosphatemia that can lead to severe weakness, malaise, and anorexia. Common side effects associated with these antacids include diarrhea (Mg) and constipation (aluminum). When antacids containing aluminum and magnesium are taken together, constipation and diarrhea may be avoided.

**Sodium bicarbonate** reacts rapidly with HCl to form water, carbon dioxide, and salt. Antacids containing sodium bicarbonate have high amounts of sodium. Therefore, in patients with hypertension or fluid overload, sodium-containing antacids can result in significant sodium retention.

**Calcium carbonate** (TUMS) is less soluble than sodium bicarbonate; it mixes with gastric acid to produce calcium chloride and carbon dioxide. Calcium carbonate is not only useful as an antacid, but it can act as a calcium supplement in the prevention of osteoporosis. The high amount of calcium in this antacid formulation, however, may cause constipation.

## AGENTS THAT PROMOTE MUCOSAL DEFENSE

### Coating Agents

Other agents used in the symptomatic relief of peptic ulcer disease include coating agents, which provide a protective layer on the gastrointestinal epithelium. An important coating agent used to alleviate the symptoms of peptic ulcer disease is **sucralfate,** a complex salt of sucrose sulfate and aluminum hydroxide. Sucralfate has little ability to alter gastric pH. Instead, in the acidic environment of the stomach, this complex forms a viscous gel that binds to positively charged proteins and, thereby, clings adheres to the epithelial cells of the stomach, including areas of ulceration. The gel protects the stomach luminal surface, including areas of ulceration, from being degraded by acid and pepsin.

Sucralfate has poor solubility resulting in little absorption systemically. It is, therefore, devoid of systemic toxicity. Constipation is one of the few side effects. In addition, it may bind to several drugs, such as quinolone antibiotics, phenytoin, and warfarin, limiting their absorption.

**Colloidal bismuth** (Pepto Bismol) is another coating agent used in peptic ulcer disease. Bismuth salts combine with mucus glycoproteins to form a barrier that protects an ulcer from acid and pepsin damage. In addition, bismuth agents may be involved in stimulating bicarbonate secretion and mucosal prostaglandin $E_2$ secretion, thereby offering further protection from acid and pepsin degradation. Colloidal bismuth has also been found to impede *H. pylori* growth and is frequently used as part of a multidrug regimen containing antibiotics and proton pump inhibitors for the eradication of *H. pylori*-associated peptic ulcers (see below).

### Prostaglandins

Prostaglandins can be used in the treatment of peptic ulcer disease (see Chapter 40), specifically in the treatment of NSAID-induced ulcers. NSAIDs are ulcerogenic because they interrupt synthesis of prostaglandins which reduce gastric acid secretion. In addition, they enhance mucosal resistance to tissue injury by inducing bicarbonate secretion, mucus production, and blood flow.

**Misoprostol** is a prostaglandin analogue used for prevention of NSAID-induced peptic ulcers. The most frequent side effects are abdominal discomfort and diarrhea. In clinical practice, these side effects often interfere with patient compliance. In addition, misoprostol is contraindicated in women who are (or may be) pregnant, because of the possibility of generating uterine contractions resulting in abortion.

## AGENTS THAT MODIFY RISK FACTORS

### Diet, Tobacco, and Alcohol

As in the introductory case, diet therapy usually involves recommendations to avoid caffeine-containing products because of their ability to increase acid secretion. Avoiding alcohol and cigarette smoking is also advised. Excessive alcohol intake is associated with erosive gastritis and an increased incidence of peptic ulcers because of direct mucosal toxicity. Cigarette smoking is thought to decrease the production of duodenal bicarbonate and diminish mucosal blood flow, leading to a delay in ulcer healing.

### NSAIDs

Many patients with peptic ulcer disease continue to take NSAIDs because they require these medications for anti-inflammatory effects or for pain control. Switching to an NSAID that does not induce further gastrointestinal toxicity can reduce the risk of gastrointestinal complications. Clinical studies have shown that COX-2 selective inhibitors effectively suppress inflammation, and they have been shown to reduce the incidence of ulcers in individuals who need to remain on NSAIDs and are concomitantly treated with a proton pump inhibitor. As these new drugs are used more, outcome data and cost analysis studies will be important in considering the future use of COX-2 inhibitors.

A **nitric oxide-releasing NSAID** is under development. Nitric oxide (NO) helps protect the gastric mucosa via several mechanisms, including vasodilation, improved mucosal blood flow, and interference with neutrophil–endothelial adhesion. Anti-inflammatory drugs that concomitantly stimulate NO synthase or donate NO would induce less damage to the gastrointestinal mucosa and could improve the healing of an ulcer, while maintaining the anti-inflammatory properties of the NSAID.

### Treatment of *H. pylori* Infection

Elimination of *H. pylori* can lead to cure of *H. pylori*-associated peptic ulcers. Broad-spectrum antibiotics, such as **amoxicillin** or **tetracycline,** combined with **metronidazole** or **clarithromycin,** are used in the treatment of *H. pylori,* together with a proton pump inhibitor or ranitidine and bismuth citrate. Commonly used regimens include triple therapy with amoxicillin, clarithromycin and a proton pump inhibitor or quadruple therapy with tetracycline, metronidazole, a proton pump inhibitor, and bismuth.

It is possible to develop resistance after therapy for *H. pylori*. Metronidazole resistance has been reported in patients with *H. pylori* infections in the United States. Resistance to clarithromycin is less common, and this drug is often the first choice. In the introductory case, Tom was given clarithromycin, as opposed to metronidazole, for this reason.

The side effects of therapy for *H. pylori* include hypersensitivity reactions to penicillin analogues, nausea, headaches, and antibiotic-induced diarrhea caused by superinfection with *Clostridium difficile*. These effects, along with complicated dosing schedules, can lead to noncompliance. Resistance to *H. pylori* is a growing concern; therefore, it is important to recognize that antibiotic regimens will be constantly evolving.

## ■ *Conclusion and Future Directions*

Peptic ulcer disease is responsible for significant morbidity and mortality in the United States. Frequently, more than one pathophysiologic mechanism is responsible for a peptic ulcer, necessitating the use of multiple pharmacologic agents (Fig. 44-6). These agents have been designed to target the major causes of this disease by decreasing acid secretion, promoting mucosal defense, and modifying risk factors. The use of IV proton pump inhibitors, COX-2 selective inhibitors, and nitric oxide-releasing NSAIDs, and screening for the presence of cytochrome P450 polymorphisms, may allow the physician to improve and customize pharmacologic therapy for patients at risk. Improved treatment of *H. pylori* infection and the development of safer NSAIDs have the potential to decrease the overall incidence of peptic ulcer disease.

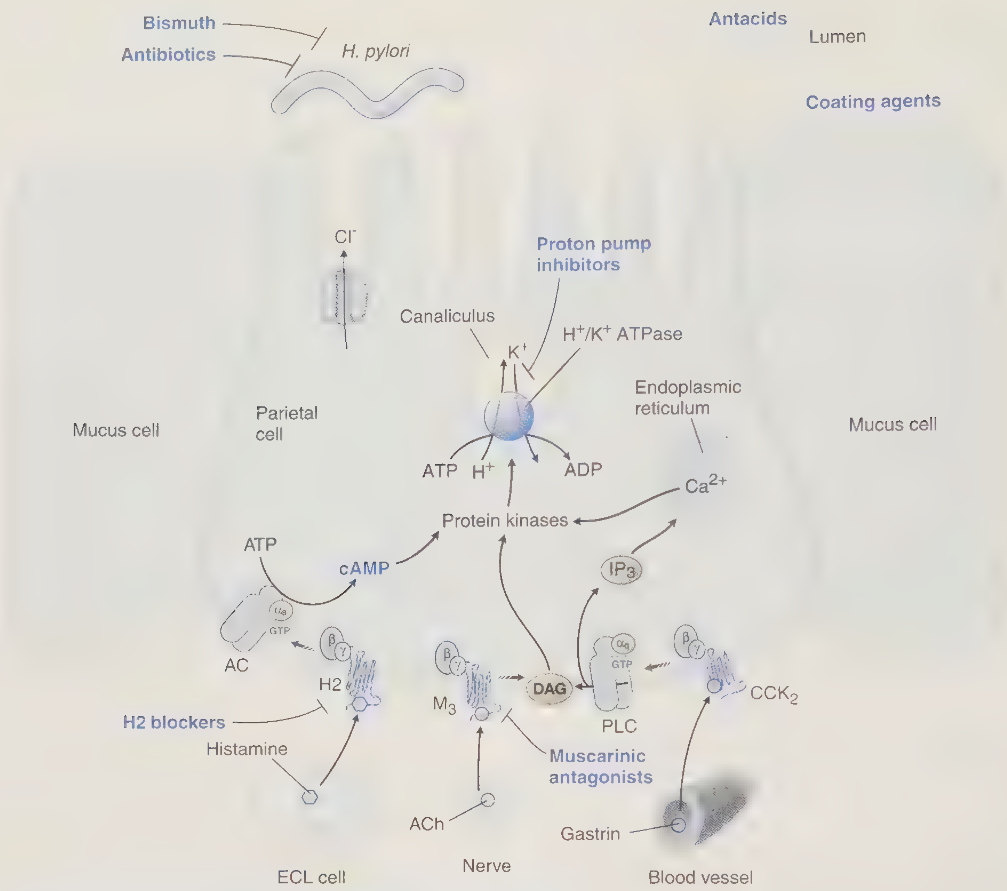


**Figure 44-6. Sites of Action of Drugs Used to Treat Peptic Ulcer Disease.** H2 blockers inhibit activation of the histamine H2 receptor by endogenous histamine. Muscarinic antagonists inhibit signaling through the $M_3$ muscarinic acetylcholine (ACh) receptor. Proton pump inhibitors decrease the activity of the $H^+/K^+$ ATPase on the canalicular membrane of the parietal cell. Antacids neutralize acid in the stomach lumen. Coating agents provide a protective layer on the epithelial surface of the gastric mucosa. Bismuth and antibiotics act to eradicate *H. pylori* from the mucus layer coating the gastric mucosa; *H. pylori* infection is an important contributing factor in the pathogenesis of peptic ulcer disease.

## *Suggested Readings*

Aihara T, Nakamura E, Amagase K, et al. Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present, and future. Pharmacol Ther 2003;98: 109–127. (*A comprehensive overview of peptic ulcer pharmacotherapy, focusing on future targets for drug development.*)

Feldman M. Gastric secretion: normal and abnormal. In: Feldman M, Friedman LS, Sleisenger MH, eds. Sleisenger & Fordtran's Gastrointestinal and Liver Disease. 7th Ed. Philadelphia: WB Saunders, 2002;715–731. (*Good review of basic GI physiology, specifically the area of gastric secretion.*)

FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001;345:433–442. (*Reviews the mechanism, pharmacokinetics, and clinical relevance of cyclooxygenase-2 inhibitors.*)

Hawkey CJ, Laine L, Simon T, et al. Incidence of gastroduodenal ulcers in patients with rheumatoid arthritis after 12 weeks of rofecoxib, naproxen, or placebo: a multicentre, randomised, double blind study. Gut 2003;52:820–826. (*Discusses advantages of COX-2 selective inhibitors compared with NSAIDs.*)

Sagar M, Tybring G, Dahl M, et al. Effects of omeprazole on intragastric pH and plasma gastrin are dependent on the CYP2C19 polymorphism. Gastroenterology 2000;119:670–676. (*Discusses the genetic component involved in treatment with proton pump inhibitors.*)

Silverstein FE, Faich G, Goldstein JL. Gastrointestinal toxicity with celecoxib vs. nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis. JAMA 2000;284:1247–1255. (*Discusses the advantages of COX-2 selective inhibitors in comparison to NSAIDs.*)

Spechler SJ. Peptic ulcer disease and its complications. In: Feldman M, Friedman LS, Sleisenger MH, eds. Sleisenger & Fordtran's Gastrointestinal and Liver Disease. 7th Ed. Philadelphia: WB Saunders, 2002:747–782. (*Good review of the pharmacology of peptic ulcer disease.*)

Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med 2002;347:1175–1186. (*Reviews the epidemiology, pathogenesis, and treatment of* H. pylori.)

Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal anti-inflammatory drugs. N Engl J Med 1999;340: 1888–1899. (*The role of nonsteroidal anti-inflammatory drugs in gastrointestinal toxicity.*)

Yeomans ND, Tulassay Z, Juhasz L, et al. A comparison of omeprazole with ranitidine for ulcers associated with nonsteroidal anti-inflammatory drugs. N Engl J Med 1998;338:719–726. (*Compares proton pump inhibitors and H2 receptor blockers in the treatment of peptic ulcer disease associated with nonsteroidal anti-inflammatory drugs.*)

**Drug Summary Table** **Integrative Inflammation Pharmacology: Peptic Ulcer Disease**

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **H2 RECEPTOR BLOCKERS**<br>*Mechanism—Decrease acid secretion by inhibiting the binding of histamine to H2 receptors on the parietal cell* | | | | |
| **Ranitidine**<br>**Famotidine**<br>**Nizatidine** | Peptic ulcer disease, gastroesophageal reflux disease (GERD) | Headache, dizziness, arthralgia, myalgia, constipation, diarrhea | Ranitidine: impaired renal function, competes for renal tubule secretion of creatinine; hepatic dysfunction | Ranitidine can be given IV to treat hypersecretory conditions or to treat patients who are not able to tolerate the oral formulation<br>Bioavailability of nizatidine is higher than that of other H2 receptor antagonists |
| **Cimetidine** | Peptic ulcer disease, GERD | Headache, dizziness, arthralgia myalgia, constipation, diarrhea, gynecomastia, galactorrhea, loss of libido | | Cimetidine reduces the cytochrome P450-mediated metabolism of certain drugs, including theophylline, warfarin, phenytoin, lidocaine, and quinidine, delaying the elimination and increasing the plasma levels of these drugs |
| **PROTON PUMP INHIBITORS**<br>*Mechanism—Decrease acid secretion by blocking the $H^+/K^+$ ATPase of the parietal cell* | | | | |
| **Omeprazole**<br>**Lansoprazole**<br>**Rabeprazole**<br>**Pantoprazole**<br>**Esomeprazole** | Peptic ulcer disease, *H. pylori* eradication, GERD, hypersecretory conditions | Headache, diarrhea, rash, nausea, constipation, abdominal pain, upper respiratory infection, anorexia, asthenia, back pain | All proton pump inhibitors: impaired renal function, impaired hepatic function<br>Omeprazole: drug interaction with ketoconazole, itraconazole due to acid environment required for absorption of these drugs | Proton pump inhibitors are metabolized in the liver by CYPC19 and CYP3A4<br>Pantoprazole can be given IV as an alternative therapy in patients who are not able to tolerate oral pantoprazole |
| **ANTACIDS**<br>*Mechanism—Neutralize gastric acid* | | | | |
| **Aluminum hydroxide** | Symptomatic relief of dyspepsia associated with peptic ulcer disease, gastritis, GERD, hiatal hernia | Phosphate depletion (severe weakness, malaise, and anorexia), constipation, osteomalacia in patients with renal failure | All antacids can potentially increase or decrease the rate or extent of absorption of concurrently administered oral drugs by changing transit time or by binding the drug | |
| **Magnesium hydroxide** | Symptomatic relief of dyspepsia | Diarrhea, hypermagnesemia (in patients with renal failure) | Same as for aluminum hydroxide | |
| **Sodium bicarbonate** | Symptomatic relief of dyspepsia; metabolic acidosis, urinary alkalinization, uric acid renal stones, diarrhea | Abdominal cramps, flatulence, alkalosis, vomiting | Same as for aluminum hydroxide; in addition, significant sodium retention in patients with hypertension or fluid overload; contraindicated in respiratory alkalosis, hypocalcemia, and hypochloremia | |
| **Calcium carbonate** | Symptomatic relief of dyspepsia; osteoporosis | Nausea, vomiting, anorexia (hypercalcemia) | Same as for aluminum hydroxide; in addition, hypercalcemia in patients with impaired renal function | |

(Continued)

**Drug Summary Table**

## Integrative Inflammation Pharmacology: Peptic Ulcer Disease *(Continued)*

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **COATING AGENTS** | | | | |
| ***Mechanism—Provide a protective layer to the gastrointestinal epithelium*** | | | | |
| Sucralfate | Peptic ulcer disease, GERD | Constipation, aluminum accumulation and toxicity (renal impaired patients) | Decreased effectiveness of quinolones (e.g., ciprofloxacin) because of chelation and decreased absorption | |
| Colloidal bismuth | GERD, peptic ulcer disease, diarrhea with associated abdominal cramps | Darkening of the tongue and/ or stool | Patients with known allergy to aspirin or other nonaspirin salicylates | Colloidal bismuth compounds are frequently used as part of a multidrug regimen for eradication of *H. pylori* because these drugs impede growth of the organism |
| **PROSTAGLANDINS** | | | | |
| ***Mechanism—Reduce basal and stimulated gastric acid secretion; enhance bicarbonate secretion, mucus production and blood flow*** | | | | |
| Misoprostol | Peptic ulcer disease (NSAID-induced) | Uterine contraction (fetal abortions), diarrhea, abdominal pain | Pregnancy | Seldom used<br>Misoprostol can cause miscarriage in pregnant patients due to the induction of uterine contractions |
| **ANTICHOLINERGIC AGENTS** | | | | |
| ***Mechanism—Decrease acid secretion by blocking muscarinic ACh receptors on parietal cells*** | | | | |
| Dicyclomine | Irritable bowel syndrome, peptic ulcer disease | Dry mouth, blurred vision, tachycardia, urinary retention, constipation, injection site irritation | Obstructive uropathy, GI obstruction, ileus, ulcerative colitis or toxic megacolon, glaucoma, myasthenia gravis | Not as effective as H2 receptor antagonists or proton pump inhibitors for the treatment of peptic ulcer disease |

# 45

# Integrative Inflammation Pharmacology: Asthma

Jeremy Tucker and Christopher H. Fanta

**Introduction**
**Case**
**Pathophysiology of Asthma**
  Airway Hyperresponsiveness
  Airway Inflammation
    *$T_H$ Cells*
    *Mast Cells*
  Relationship Between Hyperresponsiveness and Inflammation
**Pharmacologic Classes and Agents**
  Bronchodilatory Agents
    *$\beta_2$-Adrenergic Agonists*
    *Methylxanthines*
    *Anticholinergic Agents*
  Anti-Inflammatory Agents
    *Corticosteroids*
    *Inhibitors of Mast Cell Degranulation*
    *Leukotriene Pathway Modifiers*
    *Novel Immunomodulating Biotherapeutics*
  Drug Delivery and Aerosols
**Clinical Management of Asthma**
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Asthma is a multifaceted disease characterized by intermittent, reversible bronchoconstriction and chronic airway inflammation. Acquiring asthma likely requires both a genetic predisposition that is closely linked to the development of allergies and environmental exposures to repeatedly inhaled allergens. In asthmatics, a characteristic chronic, eosinophilic inflammatory response emerges in the airways: periods of increased airway inflammation are associated with heightened bronchial hyperresponsiveness; suppression of airway inflammation leads to decreased bronchial hyperresponsiveness. Some 7% of the U.S. population are thought to be asthmatics.

For many years, the treatment of asthma focused on alleviating bronchoconstriction. More recently, treatments have been directed at suppressing airway inflammation and, thereby, reducing the underlying sensitivity of the asthmatic airways—making them less vulnerable to narrowing in response to provocative stimuli.

Most people with asthma have only intermittent symptoms, despite the persistence of inflammation in their airways. However, the severity of asthma varies widely. At one extreme are Olympic and other high-performing athletes with asthma. Either because their disease is relatively mild or because their therapy is extremely effective, they can participate in world-class competitions despite their requirement for very high levels of respiratory function. At the other extreme are persons who suffer cough, wheezing, and/or shortness of breath virtually every day. Their asthma often interferes with daily activities and poses a risk for severe, potentially life-threatening, acute asthmatic attacks.

Asthma most often begins in childhood, typically before 5–7 years of age. In the United States today, asthma is the most common chronic illness of childhood and is a leading cause of school absences. The disease tends to be most severe among people living in impoverished urban communities, where individuals are more likely to be exposed to such allergenic stimuli as cockroach debris and rodent excreta, and less likely to receive adequate medical care. Both the prevalence and the severity of asthma tend to be highest in African Americans, intermediate in Latinos, and lowest in Whites, presumably because of genetic, social, and environmental factors.

## ■ Case

Ahmad is a 14-year-old student still in the sixth grade who has discipline problems at school. He plays soccer during recess on days when he's not on probation, but he often has to quit early because of difficulty breathing. Sometimes he self-administers an inhaled medication containing epinephrine, although afterward he has trouble concentrating in class because he says he feels "too nervous." When Ahmad was first diagnosed with asthma as a 6-year-old, his doctor prescribed the bronchodilator medication theophylline, one tablet to be taken twice daily. He has continued on this medicine ever since.

At home, Ahmad frequently wakes from his sleep with coughing and chest tightness. One night he experiences a severe asthma attack that he cannot control with his aerosolized epinephrine spray. His mother calls the emergency phone number, 911, as she has done many other times. Ten minutes later, Ahmad presents to the Emergency Department of the local hospital with continued difficulty breathing. He describes the sensation of a large man sitting on his chest while he is trying to breathe through a narrow straw. He has an incessant cough with thick, clear sputum. His chest examination is notable for bilateral expiratory wheezes and a prolonged expiratory phase in his breathing cycle. Laboratory tests show his total white blood cell count to be normal (8,200 cells/μL), but there is an excess of eosinophils on the differential analysis (9% eosinophils).

The emergency medicine physician gives Ahmad the fast bronchodilator albuterol, administered as an aerosol generated by a nebulizer. Ahmad's wheezing partially subsides, while at the same time he experiences a rapid pounding of his heart and tremulousness. The physician then administers an intravenous infusion of methylprednisolone, a glucocorticoid, to treat the airway inflammation. At 2-hour intervals, Ahmad receives additional nebulizer treatments of albuterol, so that, by the end of the night, he can breathe comfortably again. Upon discharge from the emergency department, his mother is given a prescription for an inhaled steroid medication, fluticasone, for Ahmad to use twice daily, as well as an albuterol inhaler to take the place of his epinephrine spray.

With his new medications, Ahmad has fewer attacks of asthma, although he continues to wake several nights per week with asthmatic symptoms. He also continues using his albuterol inhaler several times each day to relieve his cough and wheezing. He finds the steroid spray to be irritating to his throat, and he is not as faithful to its regular use as he knows he should be.

At a check-up later in the year, Ahmad sees a new doctor, who takes a special interest in his asthma. She recommends that Ahmad stop taking the theophylline tablets and use, in their place, a long-acting inhaled bronchodilator, called salmeterol, to be taken in the morning and at night. The doctor also recommends the use of a hollow plastic tube, called a spacer, to improve the delivery of the inhaled medications and minimize any irritation to the mouth and throat. The doctor also writes a note to the school nurse explaining Ahmad's new medications. She asks that the nurse actively supervise Ahmad's daily medication regimen, inform his teachers of his asthma, and help him to get tutoring to improve his reading skills.

## QUESTIONS

1. What causes Ahmad's asthma attacks?
2. How does epinephrine act to relieve his asthma symptoms?
3. Why did Ahmad become tachycardic when he received albuterol in the emergency room?
4. Why did the physician in the emergency room prescribe an inhaled glucocorticoid to be taken regularly?
5. Why did Ahmad's new primary care physician prescribe salmeterol and a spacer?
6. What other medications could the primary care physician have considered?
7. What relevance does Ahmad's asthma and its treatment have to his school performance?

## PATHOPHYSIOLOGY OF ASTHMA

There are many ways to characterize asthma. A clinician might define asthma symptomatically in terms of episodic wheezing, cough, shortness of breath, and chest tightness that varies over time and can be relieved (at least partially) by a bronchodilator drug. A pathologist might define asthma histologically as persistent airway inflammation characterized by eosinophilic infiltration and airway wall thickening or "remodeling." A physiologist might define asthma mechanistically as a condition of airway hyperresponsiveness that leads to excessive bronchial smooth muscle constriction in response to numerous different stimuli. All of these definitions can be synthesized into two recurrent themes: **airway hyperresponsiveness** (specifically, reversible bronchoconstriction) and **chronic inflammation.** Although these two pathophysiologic features of asthma are often discussed separately, their mechanisms are closely interrelated.

### AIRWAY HYPERRESPONSIVENESS

One of Ahmad's great frustrations regarding his asthma was that it would interfere with his ability to play soccer. A characteristic feature of asthma is that physical exertion precipitates constriction of the bronchial smooth muscle that surrounds the airways, thus causing widespread airway narrowing. In some people with asthma, this is the dominant stimulus that elicits symptoms; these patients are said to have "exercise-induced asthma." However, *in virtually everyone with asthma, exercise—if sufficiently vigorous—can trigger bronchoconstriction.* (In contrast, exercise has almost no effect on airway diameter in people without asthma.)

During quiet breathing, inhaled air is warmed and humidified by the nose and mouth before it reaches the lower airways. During exercise, however, the increased volume of inhaled air overcomes the ability of the upper airway to provide adequate warming and humidification. Air at essentially ambient temperature and water content penetrates deep into the lower airways, and the bronchial tubes themselves transfer heat and moisture to condition the inspired air. Increases in minute ventilation, combined with inspired air that is cold and dry, can result in significant cooling and drying of the airways.

In people with asthma, this cooling and drying of the airway walls constitute a potent stimulus to bronchoconstriction. The precise mechanism whereby this occurs in asthma is unknown; hypotheses include stimulation of mediator release from mast cells, activation of neural pathways for bronchoconstriction, and vascular dilation occurring during tissue rewarming. With a short exercise period (approximately 5 minutes or less), bronchoconstriction typically develops within a few minutes of exercise cessation. With more sustained exertion, symptoms may develop 10–15 minutes into the exercise period. Treatment with an inhaled bronchodilator such as albuterol before exercise often prevents or minimizes exercise-induced symptoms.

## AIRWAY INFLAMMATION

The chronic airway inflammation of asthma is characterized by mucosal and submucosal cellular infiltration, airway wall edema, mucus hypersecretion, airway smooth muscle hypertrophy, goblet cell hyperplasia, subepithelial collagen deposition, and epithelial cell damage. Mast cells, eosinophils, and lymphocytes are key effector cells causing this inflammatory response, which predisposes to the development of bronchoconstriction or "bronchospasm."

### $T_H$ Cells

What factors initiate airway inflammation? One hypothesis emphasizes an imbalance in the helper lymphocyte population favoring expression of the helper type-2 ($T_H2$) lymphocytes over the type-1 ($T_H1$) cells. $T_H2$ lymphocytes are identified by their release of the cytokines interleukin (IL) 4, 5, 6, 9, and 13, and are involved in allergic responses to environmental stimuli. $T_H1$ lymphocytes are identified by their release of the cytokines IL-2 and interferon gamma, and are involved in responding to infectious pathogens.

When an inhaled **allergen** is recognized by the immune system, it interacts with dendritic cells, the resident antigen-presenting cells of the airways (Fig. 45-1). Common allergens recognized by the human immune system are found in dust mite droppings, debris from cockroach bodies, mold spores, pollen granules, the saliva and skin from warm-blooded pet animals, and rodent urine. Other inhaled antigens are found in the workplace, such as flour ("baker's asthma") and plicatic acid found in certain types of sawdust ("western red cedar asthma"). Some persons with asthma are allergic to one of these allergens, others to multiple allergens, and some to no identifiable allergen at all.

Cytokines released by $T_H2$ lymphocytes play a central role in orchestrating the immune response in the airways of people with asthma. IL-4 signals B lymphocytes to produce antigen-specific immunoglobulin E (IgE). IL-4 also stimulates mast cell proliferation and the expression by vascular endothelial cells of adhesion molecules for eosinophils. IL-5 both stimulates

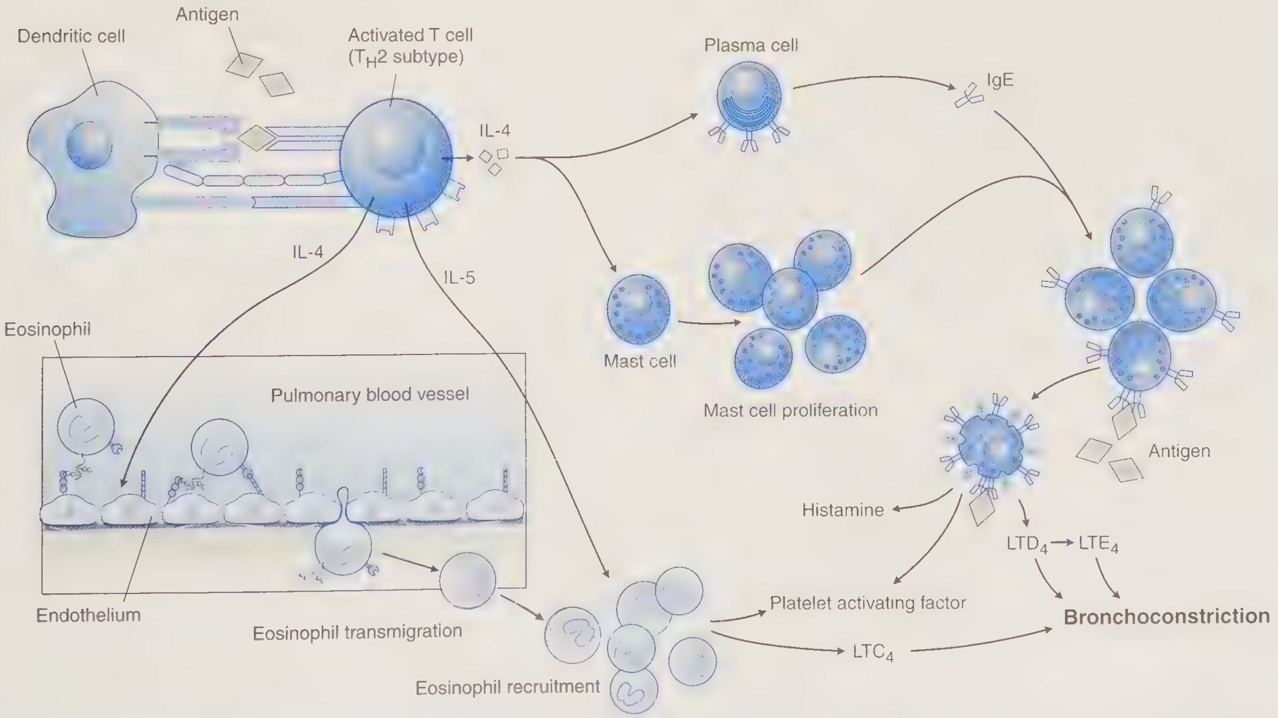


**Figure 45-1. Antigen-Induced Bronchoconstriction.** The immune system has an important role in the initiation of the allergic response that leads to bronchoconstriction in asthma. The cascade begins when dendritic cells present antigen to T cells of the immune system. These T cells (of the $T_H2$ subtype) release cytokines, especially IL-4 and IL-5, that drive the activated immune system toward an allergic response. IL-4 released by $T_H2$ cells has three primary effects. First, IL-4 promotes B cell differentiation to plasma cells that secrete antigen-specific IgE antibodies. Second, IL-4 promotes mast cell proliferation. Third, increased IL-4 up-regulates endothelial cell expression of adhesion molecules for eosinophils. $T_H2$ cells also release IL-5, which promotes eosinophil chemotaxis, transmigration, and recruitment into tissues. Activated eosinophils release such inflammatory mediators as leukotriene $C_4$ ($LTC_4$), major basic protein (not shown), and platelet activating factor.

The IgE released by plasma cells circulates in the blood and binds to IgE receptors on mast cells. The mast cells are then "primed" to release inflammatory mediators upon antigen binding to the cell-surface IgE. Antigen binding to mast cell IgE causes release of histamine, platelet activating factor, and leukotrienes. The leukotrienes released by eosinophils and mast cells are major inducers of bronchoconstriction in asthma.

the release of eosinophils into the circulation and prolongs eosinophil survival in tissue, such as in the bronchial walls.

Antigen-specific IgE molecules bind to high-affinity receptors expressed on the surface of mast cells. The mast cells are then primed, ready for repeat allergen exposure. When the same allergen is again inhaled, it binds to these IgE molecules on the surface of mast cells, leading to crosslinking of IgE receptors and cellular release of inflammatory mediators (see below).

Although it is uncertain what caused Ahmad to develop asthma, it is hypothesized that an inherited tendency to develop allergic immune responses to environmental stimuli, combined with the presence of such stimuli in his home (such as cockroach and house dust mite antigens), created the inflammatory substrate within his airways that is recognized as the basis for asthma.

### Mast Cells

Inhaled allergens are recognized by IgE molecules on the surface of mast cells, leading to mast cell degranulation and release of preformed mediators (such as histamine, platelet activating factor, and tryptase) as well as de novo synthesis of additional mediators. Most important among this latter group are the products of arachidonic acid metabolism, including thromboxanes, prostaglandins, and the cysteinyl leukotrienes (LTs), LTC$_4$, LTD$_4$, and LTE$_4$. The chemical mediators released by mast cells promote inflammation by several mechanisms. These molecules: 1) are potent bronchoconstrictors—they cause immediate airway smooth muscle contraction. They also stimulate microvascular leakage with edema formation, mucous gland secretion, and activation of sensory nerve endings (with the potential for reflex bronchoconstriction via vagal neural pathways). They promote subepithelial collagen deposition by myofibroblasts. In addition, they are chemoattractants for eosinophils, amplifying the inflammatory response by promoting tissue eosinophilia. Activated eosinophils release additional proinflammatory mediators, including major basic protein, cationic protein, eosinophil-derived neurotoxin, and additional cysteinyl leukotrienes. Proteases released by eosinophils cause epithelial damage and desquamation. In a previously sensitized individual, allergen exposure can cause both an immediate asthmatic response (bronchoconstriction) and a late asthmatic response (airway wall edema, mucus hypersecretion, cellular infiltration, and further bronchoconstriction).

## RELATIONSHIP BETWEEN HYPER-RESPONSIVENESS AND INFLAMMATION

In people experiencing a late asthmatic response to an inhaled allergen, the airways exhibit increased bronchial hyperresponsiveness for hours to days after exposure. The precise mechanism whereby asthmatic airway inflammation confers this hyperresponsiveness (and a transient period of heightened hyperresponsiveness immediately after allergen inhalation) is uncertain. One possibility is that eosinophilic inflammation causes epithelial damage and shedding, exposing nerve endings that represent the afferent limb of a bronchoconstriction reflex loop and that themselves contain proinflammatory neuropeptides (Fig. 45-2). Chemical mediators and other inhaled irritants can then provoke an exaggerated constrictor response.

A second hypothesis of the linkage between airway inflammation and bronchial hyperresponsiveness suggests that airway wall thickening internal to the smooth muscle layer leads to greater luminal narrowing for the same degree of airway smooth muscle contraction. Another mechanical explanation points to an uncoupling of the elastic pull of surrounding lung parenchyma on the airway lumen in the presence of adventitial and submucosal edema and swelling.

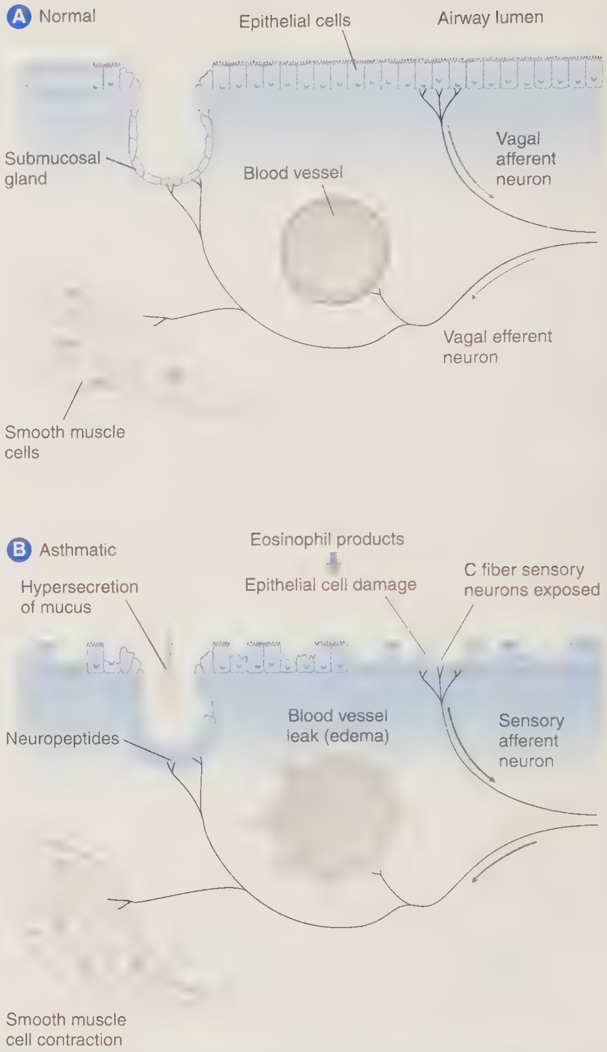


**Figure 45-2. Nociceptive Sensory Neuron Involvement in Asthma.** **A.** In individuals without asthma, airway epithelium is intact, and neural traffic from sensory nerve ending in the airways is transmitted centrally, via vagial afferent pathways. **B.** In individuals with asthma, eosinophils release products that can damage airway epithelial cells. This damage exposes C-fiber afferent sensory neurons. Neurotransmitters contained within these nerve endings include tachykinins and other neuropeptides (such as substance P, neurokinin A, and calcitonin gene-related peptide). Stimulation of the nerve endings may lead, via an antidromic or axonal (local) reflex mechanism, to release of these neurotransmitters into the airway wall, causing "neurogenic inflammation." Their local actions include increased vascular permeability and subsequent edema formation, increased smooth muscle cell contraction with consequent bronchoconstriction, and hypersecretion of mucus into the airway lumen. C-fiber stimulation can also lead to vagally-mediated reflux bronchoconstriction (via vagal afferent pathways).

According to this hypothesis, the normal bronchodilatory influence of tidal breathing and of occasional sighs is blunted by an abnormally compliant airway wall.

The importance of the association between airway inflammation and hyperresponsiveness is clear. If one can suppress the inflammatory response, either by avoiding the inciters of inflammation (such as inhaled allergens) or by treating with anti-inflammatory medications, one can reduce the propensity of asthmatic airways to develop bronchoconstriction. The therapeutic consequence is fewer asthmatic symptoms, improved lung function, and reduced risk of serious asthmatic attacks.

## PHARMACOLOGIC CLASSES AND AGENTS

The current paradigm for the ambulatory management of asthma organizes available therapies into two broad categories: **controllers** and **relievers.** Controllers (preventive medications) are taken on a regular basis to minimize the occurrence of asthmatic symptoms. Prominent in this category are anti-inflammatory medications, especially inhaled corticosteroids, which are targeted at reducing bronchial inflammation. Reliever medications are taken as needed for the rapid relief of asthmatic symptoms (and can be used preventatively before exposure to known triggers, such as exercise). β-Adrenergic agonist bronchodilators are the primary reliever medications. Other asthma medications include alternative bronchodilatory agents, inhibitors of mast cell degranulation, leukotriene pathway modifiers, and novel immunomodulating biotherapeutics.

## BRONCHODILATORY AGENTS

### $\beta_2$-Adrenergic Agonists

As previously discussed in Chapter 8, Adrenergic Pharmacology, there are two types of β-adrenergic receptors. $\beta_1$ receptors predominate in cardiac tissue, while $\beta_2$ receptors are the principal subtype expressed in bronchial tissue, particularly airway smooth muscle. *The therapeutic effect of $\beta_2$ receptor stimulation is bronchodilation.* $\beta_2$-Adrenergic agonists also cause tremor (because of stimulation of $\beta_2$ receptors on skeletal muscle), mild tachycardia, fall in blood pressure (because of stimulation of β receptors on heart and blood vessels, respectively), and a slight decrease in plasma potassium (presumably because of stimulation of a cell membrane-associated potassium–sodium pump).

By binding to their transmembrane G protein-coupled receptors, β-adrenergic agonists cause an increase in cAMP, activation of protein kinase A, phosphorylation of intracellular kinases leading to a decrease in cytosolic calcium (by either sequestration or cellular extrusion), and resultant relaxation of the smooth muscle cells (Fig. 45-3). A number of additional actions of β agonists have been observed in vitro and in vivo, reflecting the presence of β receptors on various other cell types, but these effects are of questionable clinical significance in asthma. They include decreased mediator release from mast cells, improved vascular integrity with decreased edema formation, inhibition of eosinophil activation, and increased ciliary beat frequency on airway epithelial cells.

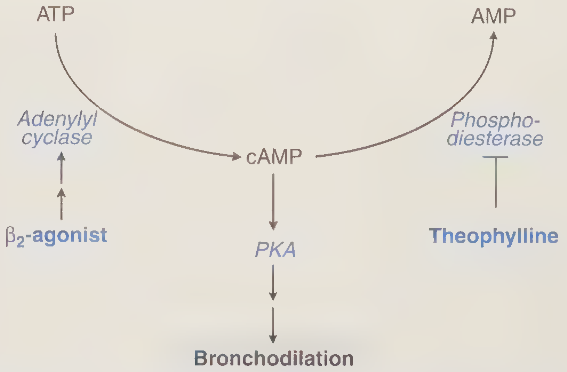


**Figure 45-3. Bronchodilatory Effects of β-agonists and Theophylline.** $\beta_2$-Adrenergic agonists activate adenylyl cyclase, increasing the production of cAMP in bronchial smooth muscle. cAMP activates protein kinase A (PKA), leading to phosphorylation of intracellular proteins and, eventually, to bronchodilation. Theophylline inhibits phosphodiesterase, also increasing cAMP in bronchial smooth muscle. Both drugs, therefore, result in bronchodilation.

People with asthma are exquisitely sensitive to β-adrenergic blockers that are not selective for $\beta_1$ receptors. Even very small doses of a nonspecific β blocker, such as propranolol or timolol, can trigger severe bronchoconstriction.

The history of the development of beta-agonist bronchodilators is of interest. **Epinephrine** (also called adrenaline) was first purified from bovine adrenal glands at the beginning of the twentieth century. It stimulates α adrenergic receptors as well as both $\beta_1$ and $\beta_2$ adrenergic receptors. As a result, in addition to causing bronchodilation, it is both a potent vasoconstrictor (and as such is used to increase blood pressure in anaphylactic shock) and cardiac stimulant (used in cardiac resuscitation). Its rapid inactivation by gastrointestinal enzymes (catechol-O-methyltransferase and monoamine oxidase) makes oral administration ineffective, but inhaled epinephrine remains available over-the-counter (as used by Ahmad in the form of Primatene Mist), and subcutaneous administration is effective therapy for acute asthmatic attacks.

**Isoproterenol** is a β-adrenergic agonist free of α-adrenergic effects. It is one of the most potent bronchodilators. It became available in the 1960s in the form of a metered-dose inhaler, the first of the convenient, pocket-sized, multidose inhalers available for administering asthma medications. It has equal $\beta_1$ and $\beta_2$ stimulatory effects, so it predictably causes considerable tachycardia and heart pounding caused by cardiac chronotropic and inotropic effects on the heart (as the result of some systemic absorption after inhalation).

The search for β-adrenergic agonists relatively selective for the $\beta_2$ receptor led to the synthesis of derivatives of isoproterenol (via modifications of the catecholamine structure). These newer, $\beta_2$ selective adrenergic agonists retain much of the potency of isoproterenol and its rapid onset of action (within 3–5 min), but have far fewer cardiac side effects. Maximal bronchodilator effect is achieved within 30–60 minutes and is sustained for up to 4–6 hours (longer than for isoproterenol). The most widely prescribed agent in this class is **albuterol;** other examples include **pirbuterol, terbutaline, metaproterenol, isoetharine,** and **bitolterol.**

Some of these molecules (for example, albuterol and terbutaline) are resistant to degradation by gastrointestinal enzymes and can be administered in tablet form. However, as expected, an orally administered β-adrenergic agonist has a slower onset of action, less bronchodilator potency, and more severe systemic effects than when the same drug is given by inhalation. The dose given by inhalation is measured in micrograms; whereas, milligram quantities are needed for oral administration.

Recently, a single isomer form of albuterol has become available as a solution for nebulization. Conventional albuterol is a racemic mixture of stereoisomers (see Chapter 1, Drug–Receptor Interactions). The active moiety is (R)-albuterol, called **levalbuterol.** At doses effecting comparable bronchodilation, purified levalbuterol causes slightly less cardiac stimulation than does conventional (racemic) albuterol.

Further modification of the structure of the β-agonist molecule (extended side chains) has led to development of the long-acting inhaled bronchodilators, **salmeterol** and **formoterol.** Both agents have a duration of action of at least 12 hours, making sustained bronchodilation with twice-daily dosing possible. Both are highly selective $\beta_2$-adrenergic agonists, with relatively few side effects. They are effective in suppression of nighttime symptoms and have proved to be potent controller medications when used in combination with such anti-inflammatory therapy as inhaled corticosteroids. Salmeterol has a relatively slow onset of action (15–20 minutes); formoterol is quick-acting, like albuterol, with its onset in less than 5 minutes. These agents are not recommended as monotherapy in asthma because they do not address the airway inflammation that underlies the disease process. People treated with long-acting β agonists alone are at greater risk of asthmatic attacks than those treated with an anti-inflammatory therapy, such as inhaled corticosteroids.

An interesting and unexplained observation regarding the use of the long-acting, inhaled β-agonist bronchodilators is that their bronchodilator potency does not diminish with repetitive use (no significant tachyphylaxis), but their bronchoprotective effect, when administered before provocative stimuli, wanes quickly with repetitive dosing. Use of the long-acting inhaled bronchodilators does not block the acute effects of the quick-reliever inhaled bronchodilators, such as albuterol.

## Methylxanthines

For many years, **theophylline** was the mainstay of bronchodilator therapy for asthma. It is orally administered in tablet or capsule form, and with slow-release formulations, it can be taken once- or twice-daily while providing 24-hour bronchodilator activity. In acute exacerbations of asthma, theophylline (and its diethylamine salt, **aminophylline**) can be administered by intravenous infusion. Like caffeine, theophylline is a methylxanthine. It has greater bronchodilator activity than caffeine (which is a very weak bronchodilator), but it shares with caffeine central nervous system stimulatory effects, including insomnia and agitation. Other common side effects of theophylline include nausea, loose stools, tachycardia, and tremor. When excessive doses are administered, serious cardiac arrhythmias and seizures can ensue.

Like β-adrenergic agonists, theophylline causes a rise in intracellular cAMP. The precise mechanism by which theophylline achieves this effect is uncertain. Postulated mechanisms have included inhibition of phosphodiesterase and stimulation of adenosine receptors (Fig. 45-3). Some anti-inflammatory effects of theophylline have been observed in vivo, including decreased airway responsiveness to histamine and inhibition of allergen-induced migration of eosinophils to the bronchial mucosa. However, its clinical use is as a bronchodilator.

Optimal dosing of theophylline is difficult. There is a large interindividual variation in the rate of hepatic metabolism of theophylline, so that the elimination half-life varies from 3–16 hours in adults. Food may affect absorption of certain formulations of the drug, and drug–drug interactions that influence clearance are common. As a result, optimal dosing requires measurement of blood levels, and caution must be exercised when prescribing medications that may decrease clearance (predominantly via hepatic cytochrome P450 isoenzymes) and, thereby, increase blood levels, such as the common antibiotics erythromycin and ciprofloxacin.

With the availability of potent, long-acting inhaled β-agonist bronchodilators, theophylline has fallen from favor for the treatment of asthma. The newer bronchodilators avoid the frequent unpleasant side effects, the need to monitor blood levels periodically, caution about drug–drug interactions, and the risk of serious adverse effects at toxic blood levels (including fatal drug overdoses) of theophylline. Research continues into the possibility of identifying phosphodiesterase inhibitors with greater bronchodilator potency and fewer systemic side effects (e.g., selective inhibitors of the isoenzyme, phosphodiesterase 4).

## Anticholinergic Agents

Inhaled **atropine** is a bronchodilator. It acts to inhibit cholinergic-induced bronchoconstriction by inhibiting the action of acetylcholine at its postsynaptic muscarinic receptor (Fig. 45-2). However, inhaled atropine is well absorbed across the respiratory mucosa, and systemic side effects are common, including dry mouth, prostatism, tachycardia, and risk of precipitating closed-angle glaucoma. In addition, mucociliary clearance of airway secretions is impaired, predisposing to airway obstruction by retained, inspissated secretions.

**Ipratropium bromide,** a quaternary ammonium salt of atropine, overcomes some of these shortcomings. It is poorly absorbed across the respiratory mucosa and does not impair the clearance of airway secretions. Inhaled ipratropium has minimal cardiac stimulatory effects.

Ipratropium, which is available both as a metered-dose inhaler and as a solution for nebulization, has been approved for use in chronic obstructive pulmonary disease (COPD) but not in asthma. In asthma, ipratropium has a slower onset (10–15 minutes) and is less potent in stimulating bronchodilation as compared to the quick-acting inhaled β-agonist bronchodilators, such as albuterol. Its duration of action is 4–6 hours, similar to albuterol. Its use in asthma is generally restricted to special circumstances: bronchoconstriction induced by the administration of beta-adrenergic blockers; acute asthmatic attacks failing to respond to inhaled beta agonists; in persons taking monoamine oxidase inhibitors (making inhaled beta agonists relatively contraindicated); and in persons with serious cardiac arrhythmias or unstable angina, where sympathomimetic stimulation should be avoided.

## ANTI-INFLAMMATORY AGENTS

Although bronchodilators are commonly used in the short-term treatment of asthma, anti-inflammatory agents are often prescribed long-term to reduce the frequency of asthmatic symptoms. These agents include inhaled corticosteroids, inhibitors of mast cell degranulation, leukotriene pathway modifiers, and novel immunomodulatory agents.

### Corticosteroids

Corticosteroids were first used to treat asthma in the 1950s, soon after their identification and purification from the adrenal cortex. Cortisone acetate and its derivatives (such as hydrocortisone, prednisolone, methylprednisolone, prednisone, and dexamethasone) proved highly effective for the treatment of severe asthmatic attacks refractory to therapy with bronchodilators. However, the myriad adverse side effects of long-term systemic steroid administration (including weight gain, growth retardation, glucose intolerance, hypertension, osteoporosis, cataracts, glaucoma, and others) mandate short-term use in all but a small percentage of people with unusually severe, refractory asthma, who are said to have "steroid-dependent asthma."

During the last two decades, corticosteroid preparations with high local activity and low systemic bioavailability have been developed for administration by inhalation. Their low systemic bioavailability reflects the large (≥ 90%) first-pass metabolism in the liver of any portion of the medication that is swallowed and absorbed from the gastrointestinal tract. **Beclomethasone dipropionate** was the first such steroid preparation to become available. When administered on a daily basis by inhalation, it effectively controlled the inflammatory component of asthma in many of the patients who previously had been dependent on systemic steroids. It achieved this outcome with only microgram quantities of drug delivered from a metered-dose inhaler, as compared to milligram amounts of orally administered steroid required for a therapeutic effect.

Because inhaled steroids can be given in small amounts with minimal systemic availability, systemic side effects are for the most part negligible. Local side effects include occasional hoarse voice (dysphonia) and risk of oral candidiasis ("thrush"), but even with long-term use, tissue atrophy and predisposition to disseminated infections do not occur.

With increasing recognition that asthma is a chronic inflammatory condition of the airways, inhaled steroids have come to be used not just in severe, "steroid-dependent" asthma but as first-line treatment for all but the mildest forms of asthma. By suppressing the inflammatory component of asthma, inhaled corticosteroids have the following beneficial effects: improved lung function, decreased bronchial hyperresponsiveness, fewer asthmatic attacks, and lower risk for the severe asthmatic attacks that can result in hospitalization or death. When taking inhaled corticosteroids on a regular basis (as their controller medication), people with asthma find that they less often need their quick-acting bronchodilator (their reliever medication) for rapid relief of symptoms.

Steroid preparations available for inhalation include beclomethasone, **triamcinolone, flunisolide, budesonide,** and **fluticasone** (the inhaled corticosteroid prescribed to Ahmad). These agents offer differences in potency (fluticasone being the most potent of the inhaled steroids) and in delivery systems (besides the traditional metered-dose inhalers with pressurized, multidose canisters, two—budesonide and fluticasone—are available as dry-powder inhalers; budesonide also comes as a liquid solution that can be made into an aerosol via nebulization). Twice-daily administration is effective and convenient; once-daily dosing has also been approved, but only in the treatment of mild asthma.

The inhaled corticosteroids are highly lipophilic and, therefore, readily cross cell membranes to enter cells. Once within the cell, the steroid molecule binds to cytosolic glucocorticoid receptors. This drug–receptor complex then enters the nucleus where it influences the transcription of target genes, in some cases causing an increase, in others a decrease in transcription. *Decreased transcription of genes coding for proinflammatory cytokines is thought to be an important mechanism of action of corticosteroids.* They also promote eosinophil apoptosis and decrease vascular leakage. Bronchial biopsies from people with asthma treated for at least several weeks with inhaled corticosteroids show more normal airway architecture, with fewer mast cells, eosinophils, and goblet cells and less epithelial cell desquamation compared with biopsies from untreated asthmatics.

Only 10–20% of the medication delivered from metered-dose inhalers is deposited onto intrathoracic airways. Most of the spray settles on the oropharynx, where it can be swallowed and absorbed systemically. As noted above, these inhaled corticosteroid preparations have a high first-pass metabolism in the liver, so that less than 10% of the swallowed medication is delivered systemically. The amount of corticosteroid medication available for absorption can be minimized in two ways: by rinsing one's mouth with water following inhaler use and by delivering medication via a spacer. Spacers are hollow chambers into which the aerosol plume from the inhaler can be directed and from which one can then promptly inhale the medication. The more uniform size and slower speed of the aerosol particles that leave the spacer maximize lower airway deposition. Larger particles from within the aerosol plume impact on the spacer walls, minimizing oropharyngeal deposition.

Nonetheless, systemic effects can be observed with regular use of *high* doses of inhaled steroids, particularly at doses exceeding 1,000 μg/day. These effects include a small risk of decrease in bone density, cataract formation, elevated intraocular pressure, and ecchymoses. In children, concern has focused on inhibition of growth. Recent studies have suggested that at conventional doses of inhaled steroids, regular use of inhaled steroids does not retard attainment of normal height.

### Inhibitors of Mast Cell Degranulation

The chromone, **cromolyn,** is an inhaled drug that inhibits the release of inflammatory mediators from mast cells. A single dose administered before exposure can blunt the response in exercise- or antigen-induced bronchoconstriction. Regular use decreases airway hyperresponsiveness and improves symptoms in persistent asthma.

The mechanism of action of the chromone seems to involve activation of a natural "turn-off switch" in mast cells. By causing phosphorylation of a cell membrane protein, mediator release is inhibited despite antigen-IgE interaction on the cell surface. Other postulated mechanisms include decreasing available intracellular calcium by blocking activation of chloride channels and possibly inhibiting neural pathways, as suggested by a decrease in the response to capsaicin via sensory nociceptive C-fibers.

Cromolyn is recommended for administration four times daily; this schedule is a significant disadvantage for a medication prescribed for long-term use. It can be delivered by metered-dose inhaler or as a solution for nebulization. Cromolyn is *not* active in relieving acute asthmatic symptoms. It is less effective than inhaled corticosteroids for symptom control and prevention of asthmatic attacks. Its major appeal, particularly in the treatment of childhood asthma, is its nearly complete absence of adverse side effects.

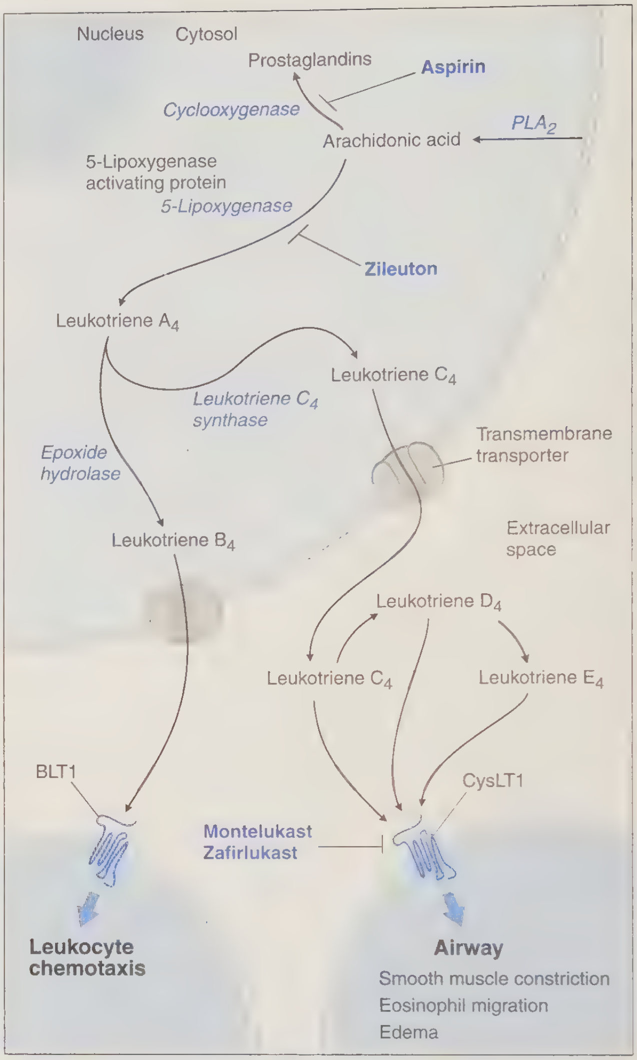


**Figure 45-4. The Leukotriene Pathway and Pharmacologic Agents that Affect Airway Inflammation.** Cytosolic phospholipase $A_2$ ($PLA_2$) releases arachidonic acid from membrane lipids. Arachidonic acid is converted either to prostaglandins by cyclooxygenase or to leukotriene $A_4$ ($LTA_4$) by 5-lipoxygenase and 5-lipoxygenase activating protein. $LTA_4$ is converted by epoxide hydrolase to leukotriene $B_4$ ($LTB_4$), which is transported across the cell membrane by a transmembrane transporter protein. $LTB_4$ acts through the BLT1 receptor as a chemotactic agent for neutrophils. $LTA_4$ is also converted to leukotriene $C_4$ ($LTC_4$), which is transported across the cell membrane. $LTC_4$ is converted to leukotriene $D_4$ ($LTD_4$), which is converted to leukotriene $E_4$ ($LTE_4$). $LTC_4$, $LTD_4$, and $LTE_4$ activate cysteinyl leukotriene receptors (CysLT1) to cause smooth muscle constriction (bronchoconstriction) and eosinophil migration. The cell types that synthesize and metabolize the leukotrienes are described in detail in Chapter 40 (see Fig. 40-5). Aspirin irreversibly acetylates cyclooxygenase, probably shunting arachidonic acid toward the leukotriene pathway; this effect may explain the finding that approximately 3–5% of asthmatic patients have exacerbation of their symptoms in response to aspirin. Zileuton inhibits 5-lipoxygenase, preventing the synthesis of leukotrienes from arachidonic acid. Zafirlukast and montelukast bind competitively to the CysLT1 receptor, inhibiting the actions of $LTC_4$, $LTD_4$, and $LTE_4$.

## Leukotriene Pathway Modifiers

The cysteinyl leukotrienes, $LTC_4$, $LTD_4$, and $LTE_4$, are potent inflammatory mediators synthesized by mast cells and eosinophils. They are 1,000-fold more potent than histamine in stimulating airway smooth muscle constriction; they also promote microvascular leakage, mucus secretion, and eosinophil chemotaxis.

Leukotrienes are synthesized from arachidonic acid via the 5-lipoxygenase pathway (Fig. 45-4). In a separate biochemical pathway, metabolism of arachidonic acid via cyclooxygenase enzymes leads to the formation of thromboxanes and prostaglandins. In approximately 3–5% of adults with asthma, use of a cyclooxygenase (COX) inhibitor such as aspirin or a nonsteroidal anti-inflammatory drug (e.g., ibuprofen, naproxen, and others) leads to a rapid (within 30–60 minutes) and often severe asthmatic attack. This reaction is not an allergic response; it is provoked by drugs with many different chemical structures and does not involve an IgE antibody response.

The precise mechanism underlying the unique susceptibility of people with aspirin-sensitive asthma is uncertain, although a characteristic finding is that they produce high quantities of the cysteinyl leukotrienes in response to administration of COX inhibitors, probably by shunting of arachidonic acid into the leukotriene pathway. One hypothesis suggests an abnormality of the promoter region of the gene for $LTC_4$ synthase, leading to the overexpression of this enzyme and, therefore, increased conversion of $LTA_4$ to $LTC_4$. Preliminary evidence suggests that the newer, selective COX-2 inhibitors, such as rofecoxib and celecoxib, are less likely to trigger asthmatic symptoms in persons with aspirin-sensitive asthma than are the nonselective COX inhibitors.

Two types of drugs have been developed that modify the leukotriene pathway: inhibitors of the 5-lipoxygenase enzyme and antagonists of cysteinyl leukotriene receptors. These medications are the first class of asthma medications to be developed as a consequence of basic science research into the immunologic basis of asthma. They are used as controller medications in asthma and have the advantages of oral administration in tablet form and no steroid-like side effects.

The lipoxygenase antagonist **zileuton** inhibits the production of the cysteinyl leukotrienes (and also of leukotriene $B_4$, a neutrophil chemotaxin). It exhibits both an acute bronchodilatory effect (indicating the importance of leukotrienes in stimulating basal bronchoconstriction in asthma) and an anti-inflammatory effect. Its short half-life dictated dosing four times daily, and its 2–4% incidence of hepatic toxicity necessitated periodic blood tests to monitor liver function. Zileuton has recently been withdrawn from the market.

Cysteinyl leukotriene receptor antagonists have been dubbed "-lukasts." **Montelukast** and **zafirlukast** have high affinity and specificity for the CysLT receptor, type 1. They are administered as tablets once- or twice-daily, respectively, and a chewable tablet has been approved for administration to children as young as 2 years old. These agents are metabolized by the liver, but hepatic toxicity has not been observed. Clinical experience suggests that some patients with asthma improve in response to treatment with leukotriene receptor antagonists, while others do not. Genetically determined differences in leukotriene production have been suspected as the cause for the observed variability in therapeutic effectiveness. Candidate gene regions have been proposed (e.g., the promoter region of the 5-lipoxygenase gene), but no practical test yet exists to distinguish "responders" from "nonresponders." A therapeutic trial of these drugs must therefore be performed in each patient.

An extremely rare complication has been described in patients newly treated with leukotriene receptor antagonists. With a frequency of approximately once in 60 million doses, patients have developed a severe inflammatory condition characterized by pulmonary infiltrates, neuropathy, skin rash, and cardiomyopathy. The common theme to this illness is a systemic vasculitis characterized by eosinophilic infiltration and granuloma formation; it is called the **Churg–Strauss syndrome.** Debate persists as to whether the leukotriene antagonists cause this vasculitis as an idiosyncratic allergic response, or whether patients with occult Churg–Strauss vasculitis have the disease unmasked when they start taking are begun on leukotriene receptor antagonists for asthma and simultaneously have their (vasculitis-suppressing) oral steroid medications withdrawn.

## Novel Immunomodulating Biotherapeutics

Leukotriene modifying drugs improve asthma by targeting one group of effector molecules released downstream in the allergic inflammatory cascade. As more of the details of the immune response in asthma have been dissected out and crucial components identified, numerous steps have been identified as potential targets for therapeutic intervention with biotherapeutic molecules. Among these targets are the interleukins released by $T_H2$ lymphocytes, especially IL-4 and IL-5, the adhesion molecules expressed on vascular endothelial cells and on eosinophils, and IgE. Novel approaches to inhibiting the activity of these molecules include specific monoclonal antibodies, soluble receptor analogues, and antisense oligonucleotides (see Chapter 49, Protein-Based and Gene Therapies).

One such agent, **omalizumab,** has recently been approved by the FDA for treatment of moderate to severe allergic asthma. Omalizumab is a **humanized monoclonal anti-IgE antibody.** The challenges involved in developing a successful biotherapeutic antibody of this sort include the following: the molecule must not bind to the IgE receptor or crosslink adjacent IgE molecules, leading to mast cell activation; it must not be recognized as an immunogen that itself elicits an antibody response; it must not form antigen–antibody complexes of potential harm to the kidneys and other organs; and it must have high affinity. Omalizumab appears to achieve these goals. It binds to the site on IgE where the IgE molecule binds to its receptor. In this way it clears unbound, circulating IgE from the circulation and prevents these molecules from interacting with their cell surface receptors. The anti-IgE monoclonal antibody, produced initially by mice, has been "humanized," meaning that 95% of the human sequence of amino acids has been substituted for the mouse sequence. This humanized monoclonal anti-IgE antibody is not recognized as foreign by the human immune system. The IgE-anti-IgE complexes are ultimately cleared from the blood without deposition in the kidney or joints.

Omalizumab is administered as an intravenous infusion or subcutaneous injection every 2–4 weeks. In clinical trials, patients randomly assigned to receive omalizumab were able to reduce their need for other asthma medications, particularly corticosteroids, with preserved or improved symptomatic well-being, more often than were patients randomized to receive the placebo. They also had fewer asthmatic attacks.

Just as biotherapeutic medicines such as TNF-α antagonists (see Chapter 43, Pharmacology of Immunosuppression) have come to play an important role in the treatment of rheumatoid arthritis and ulcerative colitis, it is anticipated that similar biotherapeutics will transform asthma therapy in the years ahead.

# DRUG DELIVERY AND AEROSOLS

Drug delivery is a particularly important issue in the treatment of asthma. Administering medication by inhalation has the virtue of maximizing local efficacy while minimizing systemic side effects. Even among patients with significant airway obstruction (low peak expiratory flow or one-second forced expiratory volume [$FEV_1$]), medication distribution is sufficiently widespread that it remains effective. Microgram amounts of medication delivered directly onto the surface of airways prove at least equally as effective as much larger amounts of medication administered orally and delivered to the airways by the systemic circulation. (The exception is in acute, severe asthmatic attacks, where systemic rather than inhaled corticosteroids are needed to reverse severe airway inflammation.)

The metered-dose inhalers widely used to deliver inhaled medications in asthma have two important drawbacks, however. First, they are difficult to use properly. Correct inhalational technique includes timing actuation to the beginning of inhalation, a slow inhalation to distribute medication widely throughout the lungs, and a brief 5–10 second breathhold at the end of inhalation to allow medication to settle onto airway surfaces before exhalation. Proper "hand–diaphragm coordination" is difficult and mistakes abound, reducing the already small fraction of dispensed medication that actually deposits onto the airways. Second, most metered-dose inhalers utilize chlorofluorocarbons (CFCs) to pressurize the medication-containing canister. Because of the detrimental effect of CFCs on stratospheric ozone, their commercial use is being banned worldwide, and they will likely be excluded from medical devices by the end of this decade.

Alternative approaches are being developed. One approach simply substitutes an "ozone-friendly" propellant for the CFCs within pressurized canisters. Hydrofluoroalkanes serve that purpose in some currently available β-agonist and corticosteroid inhalers. Another approach has focused on improved coordination between medication release and inspiration. A spring-loaded, breath-actuated device releases a metered dose of medication (the short-acting β-adrenergic agonist, pirbuterol) only when inspiratory flow is sensed mechanically by a plastic vane near the orifice of the mouthpiece. Perhaps most successful are the dry-powder inhalers. Medication is prepared as a very fine, micronized powder in individual doses (within a gelatin capsule) or as multiple doses within a single container. Medication is released and an aerosol generated by the force of a vigorous inspiration. In this manner, timing of medication delivery is perforce in synchrony with inhalation, and no propellant is necessary. Some multidose dry-powder inhalers have added a dose-counting system to the device, so that one can see precisely how many doses remain available.

Spacers can also improve inhalational technique when used in conjunction with metered-dose inhalers. As noted above in the discussion of inhaled corticosteroids, spacers are hollow chambers into which the pressurized spray from metered-dose inhalers can be directed and the medication then inhaled from the chamber. The aerosol spray exits the nozzle of the metered-dose inhaler at speeds approaching 60 miles per hour. The impact of this blast on the posterior pharynx can be uncomfortable and can interfere with efforts at a well-timed and steady inhalation. With the medication captured within the spacer chamber, these impediments to well-coordinated inspiration are overcome. The bulky size of spacers is their major drawback, hampering portability.

## CLINICAL MANAGEMENT OF ASTHMA

Pharmacologic treatment of asthma should be adjusted to the severity of symptoms and degree of impairment of lung function. A step-care approach has been recommended in the ambulatory treatment of asthma. This approach classifies patients into one of four clinical categories (Table 45-1). For patients with **mild intermittent asthma,** defined as symptoms occurring no more than twice per week, nocturnal awakenings due to asthma no more than twice per month, and lung function maintained within the normal range, as-needed use of inhaled β-agonists suffices. The β-agonists can be taken before exertion to prevent symptoms, and for relief of symptoms once they have occurred.

Patients whose symptoms occur more frequently (several times per week), who experience nocturnal awakenings several times per month, or who have normal lung function when well but decreases in peak flow up to 30% when symptomatic; that is, patients with **mild persistent asthma,** should begin regular, preventative therapy. In many instances, the drug of choice is an inhaled corticosteroid in low doses. Alternative agents include a leukotriene pathway modifier or a mast cell stabilizing drug.

Patients with daily symptoms, more than once weekly nocturnal awakenings due to asthma, or lung function below the normal range (60–80% of predicted) have **moderate persistent asthma.** A good strategy is to combine controller medications. The combination of an inhaled steroid in low doses and a long-acting inhaled beta agonist—the combination ultimately prescribed to Ahmad—has proved particularly effective. This combination provides better asthma control than high doses of inhaled steroids used alone, and it avoids the potential long-term risks associated with the systemic absorption of high-dose inhaled steroids. The combination of a leukotriene pathway modifier and an inhaled low-dose steroid also provides additive benefit as compared with either agent alone.

Finally, **severe persistent asthma** refers to patients with nearly continual symptoms and frequent nocturnal awakenings due to their asthma. Their lung function may be less than 60% of normal. In this circumstance, treatment often requires high doses of inhaled steroids combined with a second and, sometimes, a third controller medication. Occasionally, regular use of oral corticosteroids proves necessary to improve symptoms and lung function. Efforts are continually made to find the lowest dose of systemic steroids needed to provide control, sometimes using a strategy of dosing every other day, to minimize side effects.

**TABLE 45-1** Clinical Management of Asthma

| SEVERITY OF ASTHMA | CLINICAL CHARACTERISTICS | RECOMMENDED THERAPIES |
|---|---|---|
| Mild intermittent | Symptoms ≤ 2 times/week<br>Nocturnal awakenings ≤ 2 times/month<br>Lung function normal | As needed β-agonist (also to be used by individuals with persistent asthma) |
| Mild persistent | Symptoms several times/week<br>Nocturnal awakenings > 2 times/month<br>Lung function normal when asymptomatic<br>Peak flow decreased < 30% when symptomatic | Preferred: inhaled low-dose corticosteroid<br>Alternative: leukotriene pathway modifier or mast cell stabilizer |
| Moderate persistent | Daily symptoms<br>Nocturnal awakenings ≥ 1 time/week<br>Lung function 60–80% of predicted | Inhaled steroid and long-acting inhaled β-agonist |
| Severe persistent | Continual symptoms<br>Frequent nocturnal awakenings<br>Lung function < 60% of predicted | Inhaled high-dose corticosteroid and long-acting inhaled β-agonist<br>Oral corticosteroids in acute setting |

As in Ahmad's case, asthma management involves more than medication prescription. Attention to environmental exposures that provoke airway inflammation can be crucial. For some atopic persons, finding another home for the pet cat, ridding the basement of mold, or exterminating cockroaches can produce major benefits. Eliminating environmental tobacco smoke also has been shown to reduce symptoms and the frequency of asthmatic attacks in children whose parents or caregivers are cigarette smokers.

As in any chronic illness, being attentive to reasons for medication nonadherence is also important. Barriers that may prevent patients from taking their medications (or giving medications to their children) as instructed by their doctors include psychosocial issues such as depression or alcoholism; impaired comprehension of the instructions; fear of side effects from the medications; the expense of purchasing the medications; and excessively complicated multidrug treatment regimens. Fortunately, Ahmad encountered a physician who was willing to address some of the broader problems that Ahmad faced at home and school. This physician worked to ensure that these problems would not undermine management of Ahmad's asthma and that asthma and its treatments would not interfere with Ahmad's performance in school.

## Conclusion and Future Directions

This chapter illustrates issues of drug choice, delivery, combination, and adherence as they relate to a chronic inflammatory disease. Although the symptoms of asthma are often sporadic and relate in large measure to airway smooth muscle constriction, the underlying substrate of chronic allergic-type inflammation confers upon asthmatic airways their hyperresponsiveness and tendency for excessive narrowing. Understanding these pathophysiologic mechanisms for airway constriction in asthma has led to the development of more effective preventive strategies. The dual strategies of avoiding the stimuli for asthmatic inflammation and treating with effective and safe anti-inflammatory medications have succeeded in reducing the number of episodes of airway narrowing. Patients feel better, have improved lung function, need relief from quick-acting bronchodilators less often, and are better protected from severe asthmatic attacks.

Still, current asthma therapies are far from optimal. Inhaled medications can be difficult to use effectively. Doubts remain regarding the long-term safety of inhaled corticosteroids, especially in young children. Some patients continue to suffer persistent asthmatic symptoms despite available treatments. No therapy has yet been shown to modify the long-term natural history of chronic asthmatic inflammation, which, in a subset of patients, results in permanent airflow obstruction. The coming decade will offer a variety of new types of therapies for the treatment of asthma and related allergic diseases. A new era of biotherapeutics will continue to emerge that targets specific molecular steps in the immunological processes of asthmatic inflammation. As a result, the prospect for major progress in asthma pharmacology is bright.

## Suggested Readings

Barnes PJ, Adcock IM. How do corticosteroids work in asthma? Ann Intern Med 2003;139:359–370. (*Reviews molecular and cellular mechanisms of corticosteroid action in asthma.*)

Busse WW, Lemanske RF. Advances in immunology: asthma. N Engl J Med 2001;344:350–362. (*Overview of the immunologic basis for asthmatic inflammation, including helpful illustrations.*)

Drazen JM. Treatment of asthma with drugs modifying the leukotriene pathway. N Engl J Med 1999;340:197–206. (*Explanation of leukotriene-modifier therapy.*)

Leflein JG, Szefler SJ, Murphy KR, et al. Nebulized budesonide inhalation suspension compared with cromolyn sodium nebulizer solution for asthma in young children: results of a randomized outcome trial. Pediatrics 2002;109:866–872. (*Compares the two nebulizable controller asthma medications approved for use in infants and young children.*)

McFadden ER Jr. Acute severe asthma. Am J Respir Crit Care Med 2003;168:740–759. (*Reviews epidemiology, natural history, and treatment of asthmatic attacks.*)

Milgrom H, Fick RB, Su JO, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. N Engl J Med 1999;341:1966–1973. (*Results of a clinical trial showing clinical efficacy of the anti-asthma therapy, monoclonal anti-IgE antibody.*)

Naureckas ET, Solway J. Mild asthma. N Engl J Med 2001;345:1257–1262. (*Mechanisms and management of mild asthma.*)

Nelson HS. Beta-adrenergic bronchodilators. N Engl J Med 1995;333:499–506. (*Overview of $\beta_2$-adrenergic bronchodilator therapy.*)

Rachelefsky G. Exacerbations of asthma in children: the role of systemic corticosteroids. Pediatrics 2003;112:382–397. (*Comprehensive overview of management of acute asthma exacerbations in children.*)

Roux C, Kolta S, Desfougeres JL, et al. Long-term safety of fluticasone propionate and nedocromil sodium on bone in children with asthma. Pediatrics 2003;111:e706–713. (*Large study assessing skeletal development in children with asthma who were treated with inhaled corticosteroids.*)

Weinberger M, Hendeles L. Theophylline in asthma. N Engl J Med 1996;334:1380–1388. (*Review of the pharmacology and clinical uses of theophylline in asthma.*)

## Drug Summary Table Integrative Inflammation Pharmacology: Asthma

| Drug | Clinical Use | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **β-ADRENERGIC AGONISTS**<br>*Mechanism—Promote bronchodilation by increasing cAMP* | | | | |
| **Epinephrine** | All β-adrenergic agonists except salmeterol and formoterol can be used for:<br>Prevention of exercise-induced bronchoconstriction<br>Quick relief of bronchoconstriction | All β-adrenergic agonists can cause:<br>Tremor<br>Tachycardia<br>Hypokalemia | | Short-acting, α and β agonist; potent vasoconstrictor |
| **Isoproterenol** | | | | Equal $\beta_1$ and $\beta_2$ effects |
| **Albuterol**<br>**Pirbuterol**<br>**Terbutaline**<br>**Metaproterenol**<br>**Isoetharine**<br>**Bitolterol** | | | | Short-acting $\beta_2$ agonists; inhaled |
| **Levalbuterol** | | | | (R) isomer of albuterol; may cause less cardiac stimulation than albuterol |
| **Salmeterol**<br>**Formoterol** | Long duration / controller medication | | | Long-acting $\beta_2$ agonists; inhaled<br>Salmeterol has longer time to onset, while formoterol is fast-onset |
| **METHYLXANTHINES**<br>*Mechanism—Inhibit phosphodiesterase, leading to ↑ cAMP and therefore to bronchodilation* | | | | |
| **Theophylline** | Additive therapy | Tremors, insomnia, nausea<br>Narrow therapeutic index | Drug–drug interactions may ↑ or ↓ levels | Difficult to achieve optimal dosing; must monitor blood levels, due to narrow therapeutic index<br>Overdose can result in seizures and arrhythmias |
| **ANTICHOLINERGICS**<br>*Mechanism—Antagonists at muscarinic receptors; inhibit bronchoconstriction* | | | | |
| **Ipratropium** | Acute asthma | Anticholinergic—dry mouth, dry eyes, etc. | | Inhaled; less potent bronchodilator than β-adrenergic agonists<br>Generally well-tolerated |

|  |  |  |  |  |
|---|---|---|---|---|
| **CORTICOSTEROIDS**<br>*Mechanism—Numerous; reduce inflammation* |  |  |  |  |
| **Beclomethasone**<br>**Budesonide**<br>**Flunisolide**<br>**Fluticasone**<br>**Triamcinolone** | Controller medication for persistent asthma | Oral candidiasis<br>Hoarse voice<br>High doses may cause systemic effects |  | Systemic glucocorticoids are used for status asthmaticus (see Chapter 26) |
| **INHIBITORS OF MAST CELL DEGRANULATION**<br>*Mechanism—Inhibit mast cell response to IgE activation* |  |  |  |  |
| **Cromolyn** | Prevention of exercise-induced bronchoconstriction<br>Controller medication for persistent asthma | Generally well-tolerated |  | Less effective than inhaled corticosteroids for symptom control and prevention of asthmatic attacks |
| **LEUKOTRIENE PATHWAY MODIFIERS**<br>*Mechanism—Inhibit leukotriene receptor activation (see Notes)* |  |  |  |  |
| **Zafirlukast**<br>**Montelukast** | Controller medication for persistent asthma | Vasculitis (rare) |  | Zafirlukast and montelukast are antagonists of the cysteinyl leukotriene receptor<br>Used as alternative or additive to inhaled steroids in persistent asthma |
| **IMMUNOMODULATING BIOTHERAPEUTICS**<br>*Mechanism—See Notes* |  |  |  |  |
| **Omalizumab** | Controller medication for moderate to severe allergic asthma |  |  | Humanized monoclonal anti-IgE antibody<br>Administered as parenteral injection every 2-4 weeks |

# 46

# Integrative Inflammation Pharmacology: Gout

Ehrin J. Armstrong and Lloyd B. Klickstein

**Introduction**
**Case**
**Physiology of Purine Metabolism**
**Pathophysiology of Gout**
**Pharmacologic Classes and Agents**
  Management of Acute Gout: Suppressors of Leukocyte Recruitment and Activation
    *Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)*
    *Colchicine*
    *Glucocorticoids*
  Management of Chronic Gout: Agents That Lower Plasma Urate Concentration
    *Agents That Decrease Uric Acid Synthesis*
    *Agents That Increase Uric Acid Excretion*
    *Agents That Enhance Metabolism of Uric Acid*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Gout is a uniquely human disease. Most mammals possess uricase, an enzyme that metabolizes purine breakdown products into a freely water-soluble substance, allantoin. Humans, in contrast, excrete most purines as sparingly soluble uric acid. High plasma levels of uric acid can lead to deposition of uric acid crystals in joints, most frequently the first metatarsophalangeal (great toe) joint. Acute attacks of gout cause intense pain, but usually occur infrequently. A number of rational therapies exist for the treatment of gout, including drugs that suppress the immune response to crystal deposition, agents that limit the extent of inflammation, agents that reduce uric acid synthesis, and agents that increase the renal excretion of uric acid. These pharmacologic interventions provide effective therapy for most cases of gout.

## Case

One morning, Mr. Jones awakens with excruciating pain in his great toe. Even the weight of the bedsheet is enough to make him want to scream; he is unable to put on a sock or shoe. Worried that something terrible has occurred, Mr. Jones rushes to his doctor. On examination, the physician diagnoses an acute attack of gout, based on the physical findings and history. The physician prescribes ibuprofen, which relieves the pain after 2 days. Mr. Jones is well until 5 years later, when the symptoms recur and he treats himself with ibuprofen, successfully. Since then, Mr. Jones has learned to anticipate the attacks, which have slowly increased in frequency, so that they now occur roughly once weekly. He uses ibuprofen at the first hint of pain. The latest attack began last night and, having run out of ibuprofen, Mr. Jones goes to his doctor today.

Focused examination reveals a swollen, red and warm left knee, right midfoot, and right first metatarsophalangeal joint. There are 0.5-cm mobile nodules near the olecranon bilaterally and another at the inferior pole of the right patella. The rest of the exam is unremarkable. The physician aspirates Mr. Jones' left knee, revealing a cloudy yellow fluid that, on microscopic exam, contains numerous leukocytes. Abundant needle-shaped microscopic crystals are also seen, some of them intracellular. An x-ray film of the left knee is normal except for the presence of an effusion; a film of the right foot shows an erosion of the distal first metatarsal.

Mr. Jones is treated with colchicine for 3 days and naproxen for 2 days. His condition improves rapidly. Three weeks later, he returns to his physician while feeling well. He is given a prescription for allopurinol to take continuously, and one for colchicine to take during the first few months of allopurinol therapy.

## QUESTIONS

1. Why was ibuprofen effective for most of Mr. Jones's acute attacks of pain?

2. How do ibuprofen and colchicine reduce the inflammatory response during an acute attack of gout?
3. How does allopurinol act? Will it alter the frequency of Mr. Jones's painful attacks?
4. Why did Mr. Jones take colchicine during the first few months of treatment with allopurinol?

## PHYSIOLOGY OF PURINE METABOLISM

Gout is a disease caused by imbalances in purine metabolism. To understand the cause and treatment of gout, it is necessary to recall the principles of nucleotide biochemistry. Although pyrimidines such as cytosine, thymidine, and uracil are straightforward for the body to metabolize and excrete, the metabolism of purines (most notably the nucleotides guanine and adenine) is challenging. The intermediates of purine metabolism are toxic, necessitating tight regulation of purine synthesis and degradation. Furthermore, the final breakdown product of purine metabolism is uric acid, which is barely soluble in blood or urine. Increased plasma levels of uric acid are the strongest risk factor for gout, although, for poorly understood reasons, not everyone with high plasma urate develops gout.

Purines are synthesized via two general pathways: **de novo synthesis** and the **salvage pathway** (Fig. 46-1). The first step in the de novo pathway is the reaction of phosphoribosylpyrophosphate (**PRPP,** a ribose sugar with two pyrophosphates attached) with glutamine. PRPP provides the ribose sugar as one precursor for the nascent nucleotide. Hydrolysis of the pyrophosphate in a later step makes the de novo pathway irreversible. Glutamine is the precursor for inosine monophosphate, a common precursor for adenine and guanine. The reaction of PRPP with glutamine is catalyzed by the enzyme amidophosphoribsyltransferase (**amidoPRT**). AmidoPRT is activated allosterically by high levels of PRPP; PRPP is thus both a substrate and activator of amidoPRT. In general, *the cellular level of PRPP is the most important determinant of de novo purine synthesis.* High PRPP levels result in enhanced de novo purine synthesis, whereas low PRPP levels decrease the rate of synthesis.

The salvage pathway is the second important mode of purine synthesis. The first step in the salvage pathway is catalyzed by the key regulatory enzyme hypoxanthine/guanine phosphoribosyltransferase (**HGPRT**). HGPRT transfers PRPP to either hypoxanthine or guanine, resulting in the formation of inosine monophosphate (IMP) or guanosine monophosphate (GMP), respectively. Nucleotide interconversions can then yield ATP and GTP.

Increased activity of the salvage pathway has two important consequences. First, increased scavenging activity depletes cells of PRPP, thus decreasing the rate of de novo purine synthesis. Second, the salvage pathway leads to the generation of more ATP and GTP. Increased levels of these nucleotides inhibit amidoPRT in a feedback manner, resulting in a decrease in de novo purine synthesis.

Although purines can be synthesized by these two interrelated pathways, *degradation occurs via a convergent mechanism* (Fig. 46-1). Adenosine monophosphate (AMP) is deaminated, dephosphorylated, and deribosylated, forming hypoxanthine. Guanosine monophosphate (GMP) is also deaminated, dephosphorylated, and deribosylated, forming hypoxanthine. Hypoxanthine is moderately soluble and is oxidized to xanthine. Thus, xanthine is the common product of purine metabolism. A further oxidation step converts xanthine to uric acid. The enzyme **xanthine oxidase** catalyzes oxidation of hypoxanthine to xanthine and xanthine to uric acid.

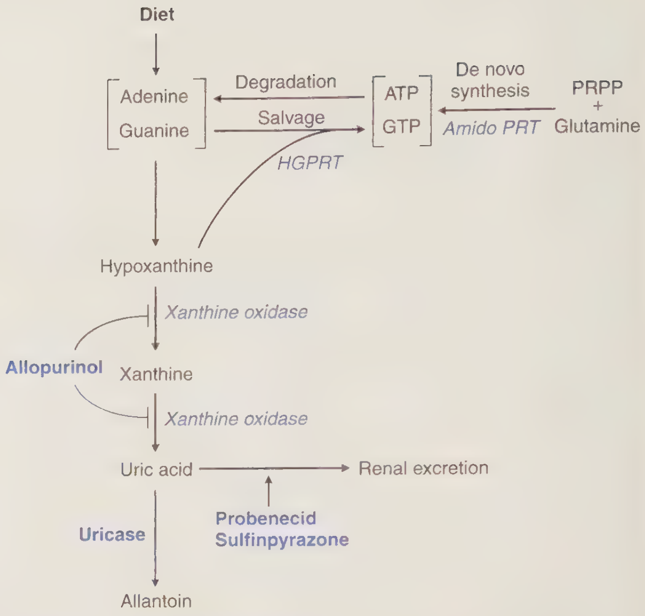


**Figure 46-1. Purine Metabolism.** Purines (adenine and guanine) can be formed via de novo synthesis or dietary salvaging. The de novo pathway utilizes the amino acid glutamine and phosphoribosylpyrophosphate (PRPP), in a reaction catalyzed by amidophosphoribosyltransferase (amidoPRT). The salvage pathway converts dietary guanine or adenine to nucleotides. Hypoxanthine–guanine phosphoribosyltransferase (HGPRT) phosphorylates and ribosylates dietary adenine and guanine, creating the purine nucleotides used for DNA and RNA synthesis. Degradation converts all purines to xanthine and ultimately uric acid, which is excreted by the kidneys or gastrointestinal tract (not shown). Pharmacologic interventions that reduce plasma urate consist of reducing urate synthesis (allopurinol and its metabolite oxypurinol), increasing urate excretion (probenecid and sulfinpyrazone), or converting urate to the more soluble allantoin (uricase).

Cross talk between the de novo and salvage pathways is important for overall regulation of purine metabolism. The de novo pathway is the most important generator of purine breakdown products. *High de novo pathway activity increases purine turnover, resulting in higher plasma uric acid concentrations.* In contrast, increased salvage pathway activity leads to decreased de novo synthesis and reduced plasma uric acid levels.

The importance of cross talk in purine metabolism is demonstrated by inherited enzyme disorders. For example, certain genetic polymorphisms that increase PRPP synthase activity lead to increased intracellular levels of PRPP: because PRPP activates amidoPRT, high levels of PRPP cause greater de novo purine synthesis, leading to increased turnover and degradation of purines and to increased plasma levels of uric acid. Similarly, genetic deficiencies of HGPRT (the critical enzyme in the salvage pathway) result in decreased salvage pathway activity and increased de novo purine synthesis and degradation, leading to increased uric acid levels. The inherited absence of HGPRT is especially crippling, resulting in the **Lesch–Nyhan syndrome,** characterized by self-mutilation, mental retardation, and hyperuricemia. Partial defects in HGPRT (i.e., polymorphisms of the HGPRT gene resulting in decreased HGPRT synthesis or activity) are thought to explain some cases of inherited gout (see below).

Uric acid is eliminated by the kidney (65%) and the gastrointestinal tract (35%). Uric acid is filtered and secreted by the kidney by the same mechanisms that process other organic anions. Approximately 90% of filtered uric acid is reabsorbed: only 10% appears in the urine. Renal excretion is important for maintenance of plasma uric acid levels; renal failure often leads to high plasma urate levels.

## PATHOPHYSIOLOGY OF GOUT

The likelihood of developing gout correlates strongly with increased plasma uric acid levels. Uric acid is a weak acid (pKa = 5.6); at physiologic pH, 99% of plasma uric acid is in the ionized, urate form. Normal urate concentration in human plasma is 4–6 mg/dL, reflecting a balance of urate synthesis, breakdown, and excretion. Urate is sparingly soluble: the plasma becomes saturated if urate levels exceed 6.8 mg/dL. A plasma level over 7.0 mg/dL for men or 6.0 mg/dL for women is classified clinically as hyperuricemia. The gender difference may be attributable to differences in urate excretion between women and men.

*Any variable that decreases the solubility of urate can result in urate crystal deposition.* Gout is most commonly seen in peripheral joints. Urate is less soluble at lower temperatures, which may explain the peripheral distribution of urate crystals. Also, joint synovial fluid is more acidic than blood, favoring crystal formation.

The pathogenesis of gout is thought to reflect deposition of urate crystals in the periarticular fibrous tissue of synovial joints after years of hyperuricemia. However, it is possible to develop gout without hyperuricemia (e.g., due to preferential deposition of urate in synovial fluid or to an immune response to urate).

The natural history of gout occurs in four stages (Table 46-1). First, asymptomatic hyperuricemia develops, either because of increased purine breakdown or decreased excretion of urate. Because most cases of hyperuricemia never develop into gout, there is no indication for treatment of hyperuricemia in the absence of gout. It is, however, important to determine the cause of severe hyperuricemia: such causes can induce a lymphoma (increased purine turnover) or renal failure (decreased excretion of urate).

For those patients with symptomatic gout, the second phase involves an acute attack of arthritis. Typically, this involves rapid onset of acute pain in a single joint, as occurred in Mr. Jones. More than 50% of gout patients have their first attack of gout in the first metatarsophalangeal joint (pain at this site is referred to as **podagra**), and almost all patients have podagra at some point once gouty arthritis develops. Without treatment, an acute attack of gout may last for hours to days, but typically resolves spontaneously. It is understood neither what causes the periodic onset of gout attacks, nor why these attacks resolve spontaneously.

The end of an attack leads to the third, intercritical phase, characterized by hyperuricemia without acute symptomatic gout. Some individuals will experience only one acute attack of gout and remain in the intercritical phase for long periods or for the remainder of their lives. Five years after his initial attack, Mr. Jones developed chronic, recurrent attacks of gout, the fourth phase. Typically, these attacks become polyarticular and more severe. Chronically high levels of plasma urate can also lead to deposition of urate crystals around synovial joints, called **tophi.** Tophi can stimulate an inflammatory response, eventually destroying the synovial lining and cartilage.

A working model for the inflammatory reaction in the joint has been proposed (Fig. 46-2). Central to this model is the phagocyte response to urate crystal deposition in the synovial fluid and synovium. Urate crystals in the synovial fluid cause complement activation and, initially, monocyte phagocytosis of the crystals. Release of chemotactic factors such as C5a and IL-8 recruit other inflammatory cells. Neutrophils release lysosomal enzymes and previously phagocytosed urate crystals into the joint, recruiting additional neutrophils.

**TABLE 46-1** Natural History of Gout

| STAGE | FEATURES | PHARMACOLOGIC INTERVENTION |
|---|---|---|
| 1. Asymptomatic hyperuricemia | Plasma urate > 6.0 mg/dL in women, > 7.0 mg/dL in men | None |
| 2. Acute gout | Acute arthritis<br>Usually 1st metatarsophalangeal joint<br>Excruciating pain | NSAIDs<br>Colchicine<br>Glucocorticoids |
| 3. Intercritical phase | Asymptomatic hyperuricemia<br>10% may never have another acute attack | None |
| 4. Chronic gout | Hyperuricemia<br>Development of tophi<br>Recurrent attacks of acute gout | Allopurinol<br>Probenecid<br>Sulfinpyrazone |

Note that the degree of hyperuricemia correlates with the likelihood of developing gout, but it is possible to develop gout without hyperuricemia. No pharmacologic intervention is indicated for asymptomatic hyperuricemia, but the cause should be investigated.

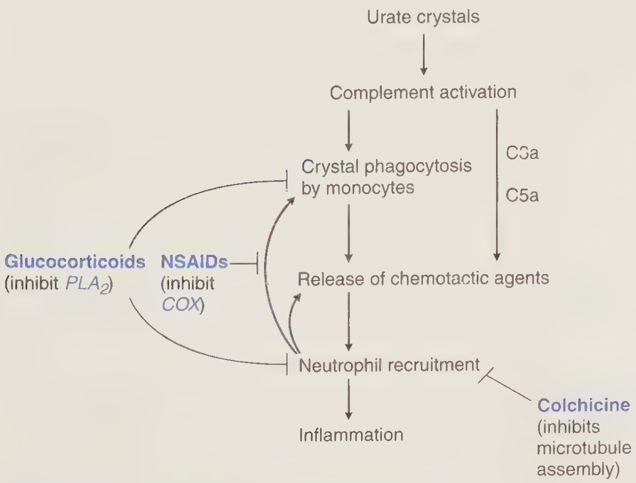

Urate crystals
Complement activation
C3a
C5a
Crystal phagocytosis by monocytes
Glucocorticoids (inhibit PLA2)
NSAIDs (inhibit COX)
Release of chemotactic agents
Neutrophil recruitment
Colchicine (inhibits microtubule assembly)
Inflammation

**Figure 46-2. Inflammation in a Gouty Joint.** During an acute attack of gout, urate crystals in the synovial fluid and synovial tissue activate complement. Complement activation leads to phagocytosis of opsonized crystals by monocyte/macrophages and release of chemotactic agents such as C5a and C3a. Monocyte-secreted factors and other chemotactic agents stimulate neutrophil recruitment, which leads to a positive feedback loop involving IL-8 and leukotriene $B_4$ release (not shown). The combination of these factors constitute the inflammatory response typical of acute gout. Pharmacologic interventions consist of inhibiting the inflammatory response, either by inactivating monocytes and neutrophils (glucocorticoids and colchicine) or by decreasing the levels of inflammatory mediators released (glucocorticoids and NSAIDs).

These processes cause the intense pain characteristic of an acute attack of gout, while the chronic inflammatory response to urate crystals can cause cartilage destruction.

# PHARMACOLOGIC CLASSES AND AGENTS

There are two main strategies for the treatment of gout: (1) management of acute attacks of gouty arthritis; and (2) long-term management of chronic gout. Although some of the same drugs are used in both acute and chronic gout, the aims of therapy differ substantially. The goal of acute gout management is to control pain using drugs that limit joint inflammation. Pharmacologic agents for the treatment of chronic gout modify either the increased production of urate or the decreased renal clearance of urate. Therapy of the chronic disease, therefore, aims to modify purine metabolism to achieve normal concentrations of plasma urate.

## MANAGEMENT OF ACUTE GOUT: SUPPRESSORS OF LEUKOCYTE RECRUITMENT AND ACTIVATION

### Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Metabolites of arachidonic acid have play an important role in the joint inflammatory response to urate crystals. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase and thereby inhibit prostaglandin and thromboxane synthesis (see Chapter 40, Pharmacology of Eicosanoids). These drugs were effective for most of Mr. Jones's acute attacks of gout; his pain responded well to ibuprofen. Clinically, **indomethacin** is one of the most often used NSAIDs for attacks of gout. The choice of an NSAID or colchicine (see below) for treatment of acute gout is generally based on the side effect profile. The serious side effects of NSAIDs include bleeding, salt and water retention, and renal insufficiency. COX-2 specific inhibitors are a promising new treatment for the management of acute attacks of gout because they may decrease the risk of gastrointestinal bleeding (see Chapter 44, Integrative Inflammation Pharmacology: Peptic Ulcer Disease).

### Colchicine

**Colchicine** binds to tubulin, inhibiting its polymerization and preventing the formation of microtubules. Administration of colchine inhibits cell division because microtubules are critical for alignment and separation of chromosomes during mitosis (see Chapter 32, Pharmacology of DNA Replication and Mitosis). Microtubules are also essential in intracellular trafficking. The effects of colchicine on neutrophils in the joint, decrease the inflammatory response. These actions include: (1) decreased trafficking of phagocytosed particles to lysosomes; (2) decreased release of chemotactic factor; (3) decreased motility and adhesion of neutrophils; and (4) decreased tyrosine phosphorylation of neutrophil proteins, with a resulting decrease in leukotriene $B_4$ synthesis.

Colchicine causes several important side effects. Epithelial cells of the gastrointestinal tract replicate quickly, and administration of colchicine inhibits their turnover; thus, diarrhea is a common complication of moderate or high doses of colchicine. Colchicine is myelosuppressive, particularly in high doses or if used concomitantly with other myelosuppressive agents, such as ganciclovir or azathioprine. At low doses, colchicine can be administered as a prophylactic therapy for chronic gout, to inhibit the occurrence of acute attacks. Drugs that alter urate homeostasis are often coadministered initially with colchicine to avoid precipitating an acute attack of gouty arthritis (see below).

Colchicine undergoes extensive enterohepatic recirculation, mediated by the liver multidrug–resistance (MDR) protein. Other drugs that inhibit the liver MDR, such as cyclosporine and verapamil, can significantly increase the fraction of the colchicine dose that is delivered to the systemic circulation (Fig. 46-3), leading to systemic colchicine toxicity. Accordingly, the dose of colchicine should be lowered when the drug is administered concurrently with any drug known to inhibit MDR activity.

### Glucocorticoids

Glucocorticoids have powerful anti-inflammatory and immunosuppressive effects (see Chapter 26, Pharmacology of the Adrenal Cortex). Glucocorticoids inhibit numerous steps in the joint inflammatory response during an acute attack of gout. Because they have widespread adverse effects, glucocorticoids are used in the treatment of acute gout only when there are contraindications to other effective therapies. They

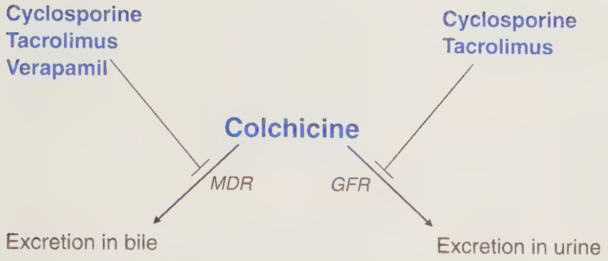


**Figure 46-3. Interaction Between Cyclosporine and Colchicine.** Cyclosporine and tacrolimus (immunosuppressant drugs frequently prescribed after organ transplantation) and verapamil ($Ca^{2+}$ channel blocker used to treat hypertension and some cardiac arrhythmias) each inhibit the activity of the multidrug-resistance (MDR) protein responsible for the hepatic excretion of colchicine. Cyclosporine and tacrolimus are also nephrotoxic, acting to reduce the glomerular filtration rate (GFR); this side effect can compromise the renal excretion of colchicine. Therefore, coadministration of colchicine with cyclosporine, tacrolimus or verapamil can lead to toxic plasma levels of colchicine.

may, however, be useful when an acute attack of gout occurs in a single joint and is unresponsive to NSAIDs or colchicine because depot preparations of prednisolone or another glucocorticoid can then be injected directly into the site of inflammation.

## MANAGEMENT OF CHRONIC GOUT: AGENTS THAT LOWER PLASMA URATE CONCENTRATION

### Agents That Decrease Uric Acid Synthesis

**Allopurinol** acts to decreases the concentration of urate in the blood. It is an example of a drug designed to inhibit a well-understood biochemical pathway. Allopurinol is a structural analogue of xanthine that inhibits xanthine oxidase (Fig. 46-4). Because of its close structural similarity to xanthine, allopurinol also acts as a substrate for xanthine oxidase. The oxidized form of allopurinol, known as **oxypurinol,** inhibits xanthine oxidase by preventing molybdenum in the active site of the enzyme from interconverting between the +4 and +6 oxidation states, essentially "freezing" the enzyme. Recall that xanthine oxidase is important for two sequential steps in purine degradation—oxidation of hypoxanthine to xanthine, and of xanthine to uric acid. Therefore, inhibiting xanthine oxidase (see Fig. 46-1) results in increased plasma levels of hypoxanthine and xanthine. Unlike uric acid, hypoxanthine and xanthine are moderately soluble in blood and can be filtered by the kidney without crystal deposition.

Allopurinol is used in the treatment of chronic gout, especially in cases caused by increased purine degradation. It should not be administered during an acute attack of gout because disruption of urate homeostasis can potentially worsen or precipitate acute attacks of gouty arthritis. Therefore, *an NSAID or colchicine is often coadministered prophylactically at the onset of allopurinol therapy to reduce the chance of precipitating an acute attack of gout.* This was the concern that prompted Mr. Jones's doctor to coadminister colchicine during the first few weeks of allopurinol therapy.

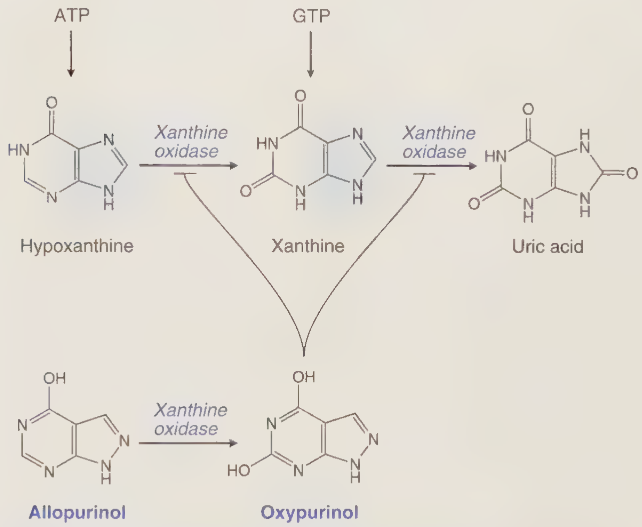


**Figure 46-4. Mechanism of Allopurinol Action.** Allopurinol is a structural analogue of hypoxanthine (similarity is highlighted in blue). Oxidation of allopurinol yields oxypurinol, a noncompetitive inhibitor of xanthine oxidase. (At low doses, allopurinol is a competitive inhibitor of xanthine oxidase.) Inhibition of xanthine oxidase decreases the production of uric acid by inhibiting two steps in its synthesis. The increased plasma levels of xanthine and hypoxanthine are tolerated because these metabolites are more soluble than urate.

Because allopurinol inhibits purine degradation, caution should be used when a patient is taking other purine analogues. For example, azathioprine and its active form 6-mercaptopurine (see Chapter 31, Pharmacology of Nucleotide Synthesis) are anticancer drugs with a purine backbone. 6-Mercaptopurine is metabolized by xanthine oxidase (Fig. 46-5). Inhibition of xanthine oxidase with allopurinol can result in toxic levels of

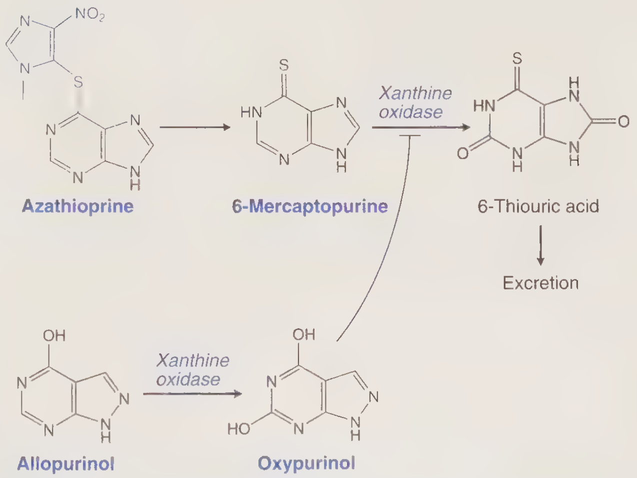


**Figure 46-5. Interaction Between 6-Mercaptopurine and Allopurinol.** 6-Mercaptopurine and azathioprine (a prodrug) are metabolized and eliminated from the body via the same pathways as other purines. Allopurinol and its metabolite, oxypurinol, inhibit xanthine oxidase, thereby inhibiting the breakdown of 6-mercaptopurine. Decreased degradation causes plasma levels of 6-mercaptopurine to rise. When coadministering 6-mercaptopurine and allopurinol (for example, in cancer chemotherapy), the dose of 6-mercaptopurine should be reduced significantly.

coadministered mercaptopurine or azathioprine because of decreased degradation of the latter drugs. Therefore, the dose of mercaptopurine or azathioprine should be reduced by approximately 75% when allopurinol is coadministered. In some cases, switching from azathioprine to a similar, but nonpurine drug, such as mycophenolic acid (see Chapter 43, Pharmacology of Immunosuppression), is another option.

## Agents That Increase Uric Acid Excretion

The kidney reabsorbs a substantial amount of filtered uric acid. Therefore, a pharmacologic agent that blocks tubular reabsorption will increase urate excretion. Such drugs are called **uricosuric agents.**

**Probenecid** was one of the first drugs used to increase urate excretion. It is an inhibitor of the basolateral anion exchanger of the proximal tubule. This exchanger acts to enhance secretion of many anions, including drugs. However, physiologic studies indicate that the proximal tubule anion exchanger mediates urate *reabsorption.* The mechanism is not known; urate reabsorption may be coupled to secretion of other anions. Therefore, inhibiting the anion exchanger increases the plasma concentration of many drugs and organic anions, such as penicillin, but *decreases* the plasma concentration of urate by decreasing urate reabsorption.

Probenecid is useful for the treatment of chronic hyperuricemia in patients with gout. Probenecid shifts the balance between renal excretion and endogenous formation of urate, thereby lowering plasma urate, dissolving urate crystals, and reversing the crystal deposition in synovial joints. Increasing renal urate excretion can predispose to formation of urate stones in the kidney or ureter. This complication can be prevented by making the urine less acidic, commonly with oral calcium citrate or sodium bicarbonate. Because uric acid has a pKa of 5.6, it will remain predominately in the more soluble neutral form if the urine pH is above 6.0. Because probenecid inhibits the secretion of most anions, the dosage should be reduced for other drugs coadministered with probenecid that are excreted by this pathway. Low-dose aspirin may antagonize probenecid action; the mechanism of this antagonism is unknown.

**Sulfinpyrazone** is a uricosuric agent that acts by the same mechanism as probenecid. It is more potent than probenecid, allowing lower effective dosing, and is effective in mild-to-moderate renal insufficiency. In addition to acting as a uricosuric, sulfinpyrazone has antiplatelet effects; it should, therefore, be given with caution to patients taking other antiplatelet agents or anticoagulants.

## Agents That Enhance Metabolism of Uric Acid

Most mammals other than humans have the enzyme **uricase,** which oxidizes uric acid to allantoin, a compound easily excreted by the kidney. Cancer chemotherapy often causes rapid lysis of tumor cells. This can result in liberation of free nucleotides and ultimately in greatly increased plasma urate levels; the elevated plasma urate is a component of the **tumor lysis syndrome.** Because of its potential for rapidly reducing plasma urate concentration, uricase has been used in the tumor lysis syndrome to prevent kidney damage associated with markedly elevated plasma urate levels and massive deposition of uric acid in tissues. (Allopurinol can also be used to prevent this component of the tumor lysis syndrome.) Currently, uricase is available in Europe as a protein purified from the fungus *Aspergillus flavus.* A small percentage of patients have allergic reactions to the foreign protein. A recombinant version of the *Aspergillus* uricase (expressed in yeast), **rasburicase,** is available in the US.

## ■ *Conclusion and Future Directions*

Disorders of purine metabolism and excretion cause gout. An imbalance between urate synthesis and excretion leads to hyperuricemia and, in some individuals, gout. The therapeutic interventions are aimed at symptomatic treatment of acute gouty attacks and, in chronic cases, decreasing plasma urate. Acute interventions seek to interrupt the cascade of inflammatory mediators by inhibiting neutrophil and monocyte activation. Treatment of chronic gout seeks to lower plasma urate by re-establishing the balance between synthesis and excretion. Allopurinol inhibits urate synthesis; probenecid increases renal urate excretion. New therapies exist for both acute and chronic gout; COX-2 selective inhibitors may reduce the side effects of chronic NSAID therapy, while recombinant uricase decreases plasma urate by converting uric acid to allantoin.

## ■ *Suggested Readings*

Emmerson BT. The management of gout. N Engl J Med 1996; 334:445–451. (*Clinical management of acute and chronic gout.*)

Schlesinger N, Schumacher HR Jr. Update on gout. Arthritis Rheumatism 2002;47:563–565. (*Review of management of refractory gout.*)

## Drug Summary Table Integrative Inflammation Pharmacology: Gout

| Drug | Clinical Uses | Side Effects/Toxicities | Interactions/Contraindications | Notes |
|---|---|---|---|---|
| **LEUKOCYTE INHIBITORS**<br>*Mechanism—See Notes* | | | | |
| Non-steroidal anti-inflammatory drugs (NSAIDs) | Acute gout | Gastrointestinal bleeding | | Inhibit cyclooxygenase (COX)<br>Indomethacin is favored agent |
| Colchicine | Acute gout | Diarrhea | ↑ levels with cyclosporine, verapamil | Acts by inhibiting microtubule assembly |
| Glucocorticoids | Acute gout | Numerous; see Chapter 26 | | Inhibit phospholipase A2, ↓ inflammatory cascade<br>Can be injected into site of inflammation |
| **INHIBITORS OF URIC ACID SYNTHESIS**<br>*Mechanism—Inhibit xanthine oxidase* | | | | |
| Allopurinol | Chronic gout | May precipitate acute gout | Increases levels of coadministered azathioprine, 6-MP | Inhibits conversion of active site molybdenum from +4 to +6 oxidation state<br>Allopurinol is also a substrate for xanthine oxidase; the product (oxypurinol) also inhibits xanthine oxidase |
| **AGENTS THAT INCREASE EXCRETION OF URIC ACID**<br>*Mechanism—See Notes* | | | | |
| Sulfinpyrazone | Chronic gout | May cause urate stones<br>Thrombocytopenia | Sulfinpyrazone and probenecid ↑ levels of penicillin and other anionic compounds | Sulfinpyrazone and probenecid inhibit renal tubule basolateral anion exchanger |
| Probenecid | Chronic gout | May cause urate stones | | |
| Rasburicase | Tumor lysis syndrome | Hemolysis<br>Methemoglobinemia | G6PD deficiency<br>Known Aspergillus sensitivity | Acts by converting urate (insoluble) to allantoin (soluble)<br>Aspergillus protein recombinantly expressed in yeast |

# VII

# Principles of Toxicology

# 47

# Principles of Toxicology

Joshua M. Galanter, Sarah R. Armstrong, Laura C. Green, and Armen H. Tashjian, Jr.

**Introduction**
**Case**
**Pathophysiology of Toxic Exposures**
  Toxicokinetics
    *Toxic Exposures*
    *Absorption*
    *Distribution*
    *Elimination*
  Toxication and Detoxication
**Mechanisms of Toxicity**
  Molecular Mechanisms of Toxicity
    *Nonspecific Macromolecular Damage*
    *Reactive Species*
    *Inflammatory and Immune-Mediated Mechanisms*
    *Enzyme and Receptor-Mediated Toxicity*
  Cellular Repair Mechanisms and Cell Death
  Organ-Directed Toxicity
  Carcinogenesis
  Teratogenesis
**Prevalent Toxic Exposures**
  Cigarette Smoke
  Ethanol
  Food Poisoning
  Lead
**Treatment for Acute Exposures**
  Principles for Treating the Acutely Poisoned Patient
  Toxicokinetic-Based Treatments
    *Prevention of Absorption*
    *Inhibition of Toxication*
    *Enhancement of Metabolism (Detoxication)*
    *Enhancement of Elimination*
  Inactivation of Poisons
    *Heavy Metal Chelators*
    *Antivenins and Antibody Binding*
  Pharmacologic Treatment
    *Pharmacologic Antagonism*
    *Pharmacologic Enhancement of Physiologic Function*
    *Active Site Restoration*
    *Metabolic Pathway Alternatives*
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

**Toxicology** is the study of deleterious effects of physical, chemical, or biological substances. The study of toxicology predates that of pharmacology, and the discovery of most pharmacologically active agents before the 20th century derived from the study of these substances as poisons. In modern times, toxicology has expanded its role to include the use of toxins for the study of enzymes and receptors. This shift in focus mirrors modern pharmacology, which is the study of not only drugs, but also their target molecules and mechanisms. In addition, toxicology now includes many public health elements, such as **occupational safety** and **environmental toxicology,** which seek to reduce environmental exposures to acceptable levels, **analytic toxicology,** the qualitative or quantitative evaluation of the presence of toxic substances, and **forensic toxicology,** the use of toxicology for legal purposes.

The field of toxicology is broad; this chapter focuses on the principles of toxicology as they pertain to the behavior of deleterious foreign substances, termed **xenobiotics.** Because the human body does not differentiate between a xenobiotic taken for therapeutic effect and a "poison," the distinction between drug and toxin is somewhat artificial. Thus, the discussion also includes the treatment of commonly overdosed therapeutics, although the mechanisms of toxicity of these therapeutics may be discussed elsewhere in this book.

## Case

The year is 1945, and Mary Price is 26 years old. She is working in a rubber factory in Cincinnati, Ohio, where she is responsible for making adhesives. During a 4-month period, Mary uses a benzene-based solvent as part of the adhesive production process. At about that time, she begins to experience increasing fatigue. One day, she accidentally tips over a barrel of benzene and inhales a large quantity of vapors. Over the next hour, she develops a severe headache, becomes dizzy, weak, and nauseated, and starts vomiting. Shortly afterward, her chest tightens, she has shallow breathing, and she falls into a coma. She is taken outside, but she remains unconscious for several hours before waking up.

Mary seems to recover completely, but remains fatigued. About a month later, she develops a severe, unremitting oral abscess. A week after the abscess appears, Mary contracts bacterial pneumonia and is admitted to the hospital. There, she is found to be profoundly anemic and has a very low white blood cell count. Mary slowly recovers. Doctors at the hospital had recently seen several cases of women exposed to benzene who developed pancytopenia. Because of this association between benzene exposure and pancytopenia, the doctors advise Mary to find work in another part of the factory.

Mary is able to find a new job, and seems to have recovered completely. However, in 1956, she again develops multiple bacterial infections, and her hematological examination shows she has leukemia. Tragically, Mary dies from overwhelming bacterial sepsis, despite her doctors' best efforts.

### QUESTIONS

1. How are compounds such as benzene absorbed and distributed to their target tissues?
2. How does benzene cause its toxic effects?
3. How do the effects of Mary's large, accidental, acute exposure to benzene differ from the effects of her chronic exposure to smaller doses of benzene?
4. Was benzene responsible for Mary's leukemia, and, if so, how did the compound cause her cancer?

## PATHOPHYSIOLOGY OF TOXIC EXPOSURES

Toxicology is the study of the interaction between poisons and living organisms. Because the body cannot distinguish between therapeutic and toxic xenobiotics, many of the concepts applicable in the study of pharmacology apply equally to toxicology. Thus, concepts such as pharmacodynamics and pharmacokinetics, covered in Chapter 2 and Chapter 3, respectively, are relevant to toxicology; toxicologists term the analogous concepts **toxicokinetics** and **toxicodynamics.**

The pathway leading from chemical exposure to end-organ toxicity involves several steps, at each of which factors may act to favor or prevent toxicity. For example, barrier functions of the skin, respiratory endothelium, and gastrointestinal epithelium may prevent absorption, or they may allow a xenobiotic to enter the circulation. Once in the circulation, the toxin may be carried to its target or may be prevented from reaching it. The toxin may be metabolized to inert products, primarily in the liver, a process termed **detoxication.** However, certain otherwise nontoxic materials may be converted into toxic metabolites, a process termed **toxication.** Finally, the toxin may be excreted or reabsorbed. Once the toxin causes tissue damage, the tissue may undergo repair or abnormal repair (discussed below). Thus, exposure to a (potentially toxic) xenobiotic is not necessarily injurious.

## TOXICOKINETICS

**Toxicokinetics** is the study of the absorption, distribution, and excretion of toxic parent compounds and metabolic products. With this information, it is possible to predict the concentration of toxin that reaches the site of injury and the resulting damage.

### Toxic Exposures

Exposures to toxins may occur as a single, large exposure or more insidiously, with repeated, smaller exposures. This is illustrated in Ms. Price's case, when she accidentally tipped over the barrel of benzene, exposing her to a large dose. **Acute toxicity** is the result of a single, large exposure to the toxic agent—effects are usually visible within minutes to hours. Occasionally, the effects of acute toxicity are not visible for weeks to months after the initial exposure. For example, Mrs. Price's pancytopenia became evident weeks after the accident. Benzene's toxicity is caused by the destruction of rapidly dividing hematopoetic stem cells, which are particularly vulnerable to phenolic metabolites of benzene. However, circulating cells are unaffected, so the toxicity that Mary experienced was not evident until peripheral cells were removed from the circulation.

**Chronic toxicity** is the effect of a toxic insult that occurs over a prolonged period of time. Often, chronic toxicity is manifested after years of exposure, sometimes long after the patient is no longer exposed to the toxin. Mrs. Price had been feeling fatigued, most likely because of anemia resulting from her chronic exposure to benzene, even though her daily exposure to benzene before the accident had been relatively low. This was a manifestation of chronic toxicity.

### Absorption

A toxin must cross an epithelial layer to be absorbed systemically. The three primary sites of absorption where this may occur are the gastrointestinal tract, the respiratory tract, and the skin. Although the epithelia of the respiratory and gastrointestinal tracts serve as a barrier, they also have an absorptive function, for oxygen and nutrients, respectively.

Ingested toxins include all toxic contaminants of food, such as **mercury** or **dioxins** in fish; **pesticides** (both natural and man-made) in produce; a majority of therapeutics; and **lead** in dirt or paint. Inhaled substances such as **asbestos** may subsequently be ingested after they are moved from the pulmonary system to the oropharynx by the mucociliary elevator function of the bronchial epithelium and trachea.

Although few therapeutics are absorbed through the pulmonary alveolar epithelium, a number of toxic exposures occur by this route. All humans breathe air pollutants such as cigarette smoke (including second-hand smoke), motor vehicle exhaust, and pollutants formed through atmospheric transformations, such as ozone. Workers in certain industries may also breathe unusually high levels of **benzene** (as in Mary Price's case), **tetrachloroethylene** (used in dry cleaning), or asbestos (formerly used in substantial quantities as a heat- and fire-resistant material). Agents of chemical warfare, such as **"nerve gases"** and **nitrogen mustards,** may also be absorbed via the respiratory tract (see Chapter 7, Cholinergic Pharmacology).

Unlike the gastrointestinal mucosa and the pulmonary alveolar epithelium, the skin is relatively impermeable and generally protects systemic tissue from surface exposures. Specifically, toxins must diffuse through seven layers of epidermis, including the **stratum corneum,** which is the rate-limiting step. However, a number of substances, such as caustic liquids, may disrupt the protective function of the skin. Others, typically organic compounds with low vapor pressure, such as **glycol ethers** and certain insecticides, can passively diffuse through the skin, especially after prolonged contact.

## Distribution

Most toxins must pass through the circulation to exert their toxic effect. This distribution is usually accomplished within seconds to minutes. The rate of distribution to a particular tissue is directly related to the blood flow to the tissue. As a consequence, vessel-rich organs such as the brain, liver, and kidney are exposed to toxins more rapidly than are fat, bone, and other tissues with low blood flow. The eventual concentration of toxin in each tissue is determined by the affinity of the toxin for the tissue, although a significant amount of redistribution may occur. Although the circulatory system provides rapid transport of absorbed toxins to their target organs, proteins in the plasma also retard distribution by binding the toxin.

Toxins reach individual cells by diffusion and enter cells by crossing the plasma membrane. Lipophilic molecules can diffuse across the cell membrane directly, whereas water-soluble molecules enter through aqueous channels or pores or by receptor-mediated mechanisms. In some cases, pumps actively transport the toxin into the cell. The toxin may also be excluded by pumps such as the **multiple-drug resistance transporter.** In the central nervous system, the **blood–brain barrier** (see Chapter 6, Principles of Nervous System Physiology and Pharmacology) also helps exclude polar molecules from the CNS.

## Elimination

Elimination of most toxins occurs analogously to most therapeutics (see Chapter 3). Renal excretion and hepatic excretion are the two most common means of elimination, although the respiratory system can also eliminate gases and volatile compounds. Elimination kinetics of toxins and therapeutics are similar, although certain toxic xenobiotics may remain in the body much longer because of the ability of tissues such as fat or bone to store such molecules and release them very slowly. For example, *lead may be stored in bone for decades following exposure, and fat may store hydrophobic toxins such as DDE* (dichlorodiphenyldichloroethylene), which is the metabolite of the once widely used pesticide DDT (dichlorodiphenyltrichloroethane), *for years.* Inhaled particulates that are phagocytosed by macrophages may never be eliminated, and can be seen in the lymph nodes of smokers and urban dwellers at autopsy.

## TOXICATION AND DETOXICATION

Like therapeutics, xenobiotics are frequently modified, usually in the liver, by phase I and phase II reactions. As discussed in Chapter 4, Drug Metabolism, these reactions increase xenobiotic hydrophilicity, thus enabling excretion. Phase I reactions, which are frequently carried out by the cytochrome P450 enzyme system, generally oxidize compounds to create polar functional groups. Phase II reactions conjugate the newly created functional groups with highly water-soluble compounds such as glucuronate, allowing the conjugate to be excreted in the urine or bile.

Like therapeutics, xenobiotics may undergo three possible changes:

- A xenobiotic can be rendered less toxic through enzymatic modification. This process is termed **detoxication.**
- A toxic xenobiotic can be converted into another toxic species.
- A relatively inert xenobioitic can be converted into a toxic species. This process is termed **toxication.**

Although a minority of therapeutics are administered in the form of **prodrugs** (inert compounds that are converted into active compounds by metabolic action), a number of xenobiotics are toxified. For example, **benzene,** to which Mrs. Price was exposed, is oxidized to its epoxide by cytochrome P450. The resultant benzene oxide then undergoes a number of enzymatic and nonenzymatic reactions that produce several toxic phenolic and polyphenolic compounds. These compounds are transported to the bone marrow, where they affect stem cells and may cause aplastic anemia. Toxication is relatively common because many phase I reactions, particularly those carried out by the cytochrome P450 system, are oxidation reactions that produce highly reactive intermediates. As discussed below, reactive species can cause significant damage to cells.

It should also be noted that the side effects of some therapeutics occur via their metabolism to toxic compounds. For example, at therapeutic doses, **acetaminophen,** an analgesic pain reliever, is sulfated or glucoronated and excreted. After large doses, the drug is converted to **N-acetylbenzo-*p*-quinoneimine** (NAPQI), a highly reactive species that can be conjugated with glutathione to produce an inert conjugate. In cases of severe overdoses, however, hepatic glutathione reserves fall, and the NAPQI is free to react with hepatic proteins and cause centrilobular necrosis and acute noninfectious hepatitis. The ultimate toxic potential of acetaminophen, and many xenobiotics, is frequently determined by the balance of competing toxication and detoxication reactions.

## MECHANISMS OF TOXICITY

Just as therapeutic pharmacologic agents act via specific mechanisms, various mechanisms of toxicity have been identified. At a molecular level, compounds can interfere with cellular and molecular functions, such as by gross physical interference with lipids, nucleic acids, and proteins; by forming species that react with biological macromolecules; by generating inflammatory or immune responses; or by stimulating or inhibiting vital cellular processes mechanistically, including oxygen delivery, energy generation, ion sequestration, neurotransmission, gene expression, and cellular replication.

Once a cell has been damaged by a toxin, it still may be able to repair the damage. If it is unable to do so, the cell may undergo **apoptosis** or **necrosis,** depending on the kind and extent of the damage. Prolonged exposure to toxins may result in **organ dysfunction, fibrosis, carcinogenesis** (the development of cancer), or **teratogenesis** (the development of birth defects).

*Toxic agents often act by more than one mechanism.* For example, a reactive species that forms a macromolecular adduct can lead to an inflammatory reaction. Other agents may cause local tissue damage in some patients but severe inflammatory reactions in others. For example, the sting venom of certain species of *Hymenoptera* (ants, bees, wasps, and hornets) causes only local tissue damage in most people. However, in certain sensitized individuals, a bee sting may result in a severe anaphylactic response. Likewise, toxins may affect multiple tissues and cause different tissue toxicities. As seen in the case of Ms. Price, benzene can cause acute respiratory depression after a large exposure, myelosuppression some time after the initial exposure, and years later, leukemia.

## MOLECULAR MECHANISMS OF TOXICITY

### Nonspecific Macromolecular Damage

Strong acids, alkalis (caustic agents), oxidants, or reducing agents damage tissue by altering the structure of proteins, lipids, carbohydrates, and nucleic acids so severely that cellular integrity is lost. These substances, such as **potassium hydroxide** in drain openers and **sulfuric acid** in car batteries, produce **chemical burns** by hydrolyzing, oxidizing, or reducing biologic macromolecules or by denaturing proteins. **Detergents** can also cause nonspecific tissue damage by disrupting and dissolving the plasma membrane of cells.

Although some of these agents may target particular macromolecules, direct tissue-damaging agents tend to be relatively nonspecific. Thus, the systems most commonly affected are those most exposed to the environment. Skin and eyes are frequently affected through direct contact, often by splashes and spills. The respiratory system is affected when toxic gases or vapors are inhaled, whereas the digestive system is affected by accidental or deliberate ingestion.

Many agents can cause damage to deep tissues after breaking through the barriers formed by the skin. Other agents are able to pass through the skin causing relatively little local damage, but destroy deeper tissues such as muscle or bone. For example, **hydrofluoric acid** (HF) causes milder skin burns than an equivalent amount of **hydrochloric acid** (HCl). However, once HF reaches deeper tissue, it destroys the calcified matrix of bone. In addition to the direct effects of the acid, the release of calcium stored in bone can cause life-threatening cardiac arrhythmias. For this reason, HF can be more dangerous than an equivalent amount of HCl.

Three characteristics determine the extent of tissue damage: the compound's identity, its strength, and its **buffering capacity,** or its ability to resist change in pH or redox potential. As mentioned above, HF is more injurious than an equivalent amount of HCl. In general, a stronger acid or base (measured by pH) or oxidant or reductant (measured by redox potential) will cause more damage than an equivalent compound at a more physiologic pH or redox potential. A solution of $10^{-2}$ M of sodium hydroxide in water has a pH of 12 but has a low capacity to cause tissue damage, because it has a small buffering capacity and is rapidly neutralized by body tissue. In contrast, a buffered solution of pH 12, such as might be found in wet ready-set concrete [made with buffered $Ca(OH)_2$], can cause more serious alkali burns because tissues cannot readily alter the material's pH.

### Reactive Species

Reactive species are **nucleophiles, electrophiles,** or **free radicals** that can react chemically with biologic macromolecules and other chemicals. Reactive species tend to be more specific in their site of action than the nonspecific agents described above and frequently show a predilection for particular tissues and macromolecules within the tissue. Most reactive species are metabolites of otherwise chemically inert compounds and cause damage in the tissues in which they are generated, which may be distant from the site of exposure. For example, **carbon tetrachloride** is a solvent formerly used as a cleaning agent and fire extinguisher, and is not hepatotoxic per se. Its metabolism is primarily in the liver, via cytochrome P450 to a trichloromethyl radical ($CCl_3\cdot$), which reacts with oxygen to form the trichloromethyl peroxy radical ($CCl_3OO\cdot$). Both the trichloromethyl radical and its peroxy derivative are reactive free radicals that can attack double bonds in unsaturated lipids, creating toxic organic free radicals. These species cause toxicity (renal toxicity, hepatotoxicity, and liver cancer).

### Inflammatory and Immune-Mediated Mechanisms

In the **inflammatory response** to tissue damage, the immune system removes cells damaged by toxins and releases immune mediators that are important in repairing tissue damage (see Chapter 39, Principles of Inflammatory and Immune Pharmacology). However, *the immune response can also be pathologic and triggered by toxins that do not themselves produce significant damage.* The two principal mechanisms by which toxins may produce damage through immune mechanisms are **hypersensitivity responses** (an allergic response) and **autoimmune reactions.**

Prior exposure to a substance is required for each of the four types of hypersensitivity reactions (see Fig. 47-1). A **type I** hypersensitivity response (**immediate hypersensitivity**) results from the production of IgE following exposure to an antigen.

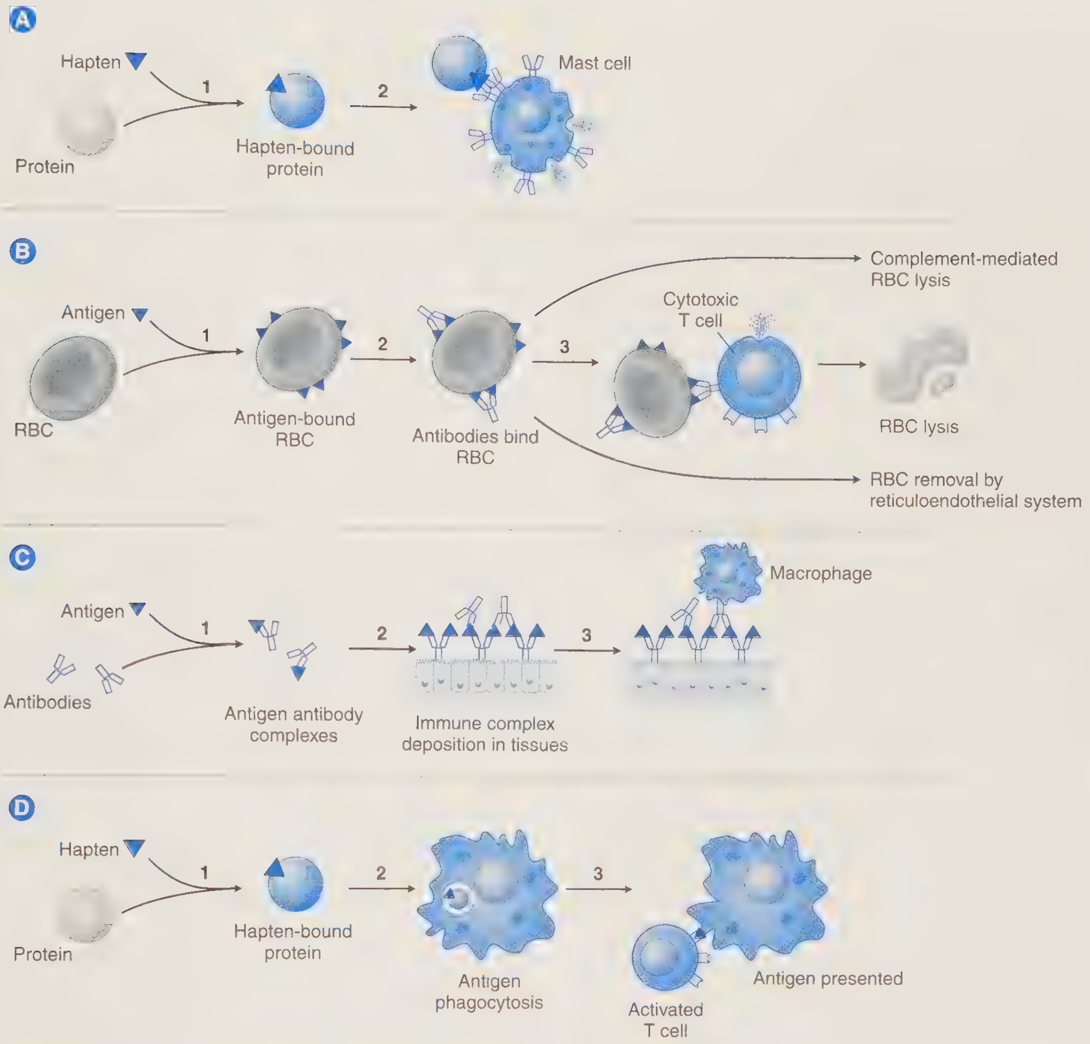

A
Hapten
Protein
1
Hapten-bound protein
2
Mast cell
B
Antigen
RBC
1
Antigen-bound RBC
2
Antibodies bind RBC
3
Cytotoxic T cell
Complement-mediated RBC lysis
RBC lysis
RBC removal by reticuloendothelial system
C
Antigen
Antibodies
1
Antigen antibody complexes
2
Immune complex deposition in tissues
3
Macrophage
D
Hapten
Protein
1
Hapten-bound protein
2
Antigen phagocytosis
3
Activated T cell
Antigen presented

**Figure 47-1. Mechanisms of Hypersensitivity Reactions (allergies).** **A.** Type I hypersensitivity reactions occur when a hapten binds to a protein (1). The antigen crosslinks IgE antibodies on the surface of a mast cell, leading to mast cell degranulation (2). Mast cells release histamine and other inflammatory mediators. **B.** Type II hypersensitivity reactions occur when an antigen binds to the surface of a circulating blood cell, usually a red blood cell (RBC) (1). Antibodies to the antigen then bind the surface of the RBC (2), attracting cytotoxic T cells (3), which release mediators that lyse the RBC. Binding of antibody to RBCs can also directly stimulate complement-mediated RBC lysis and RBC removal by the reticuloendothelial system. **C.** Type III hypersensitivity reactions occur when antibodies bind to a soluble toxin, acting as an antigen (1). The antigen/antibody complexes are then deposited in the tissues (2), attracting macrophages (3) and starting a complement-mediated reaction sequence (not shown). **D.** In a type IV hypersensitivity reaction, a hapten binds to a protein (1), which is phagocytosed by a Langerhans cell (2). The Langerhans cell migrates to a regional lymph node, where it presents the antigen to a T cell, thereby activating the T cell (3).

The antigen may be a foreign protein, such as **pet dander** or food proteins, or it may be a small molecule that reacts with a protein to form a **hapten.** Subsequent exposure to the antigen causes mast cells to degranulate, releasing inflammatory mediators such as histamine and leukotrienes that promote bronchoconstriction, vasodilatation, and inflammation. If manifest in the skin, a type I hypersensitivity response results in a **wheal-and-flare reaction.** In the upper respiratory tract, symptoms of hay fever such as conjunctivitis and rhinitis develop, while in the lower respiratory tract, asthmatic bronchoconstriction may occur (see Chapter 45, Integrative Inflammation Pharmacology: Asthma). For example, chloride salts of **platinum,** used in the mining, metallurgic, chemical, and automobile industries, are potent allergens that may cause rhinitis and asthma through a type I mechanism. Systemically, type I hypersensitivity can produce anaphylaxis, as in the case of certain insect stings mentioned above.

A **type II** hypersensitivity response (**antibody-dependent cytotoxic hypersensitivity**) results when a toxin binds to cells, usually red blood cells, and is recognized by an antibody, usually IgG. The antibody then triggers lysis of the cell by enabling complement fixation or by the action of cytotoxic T cells or phagocytosis by macrophages. Type II responses are rare side effects of a number of drugs, including penicillin and quinidine. Exposure to certain reactive nucleophilic chemicals, such as **diisocyanates** (used in the production of adhesives, paint hardeners, and coatings) and **acid anhydrides** (used in the production of specialty chemicals such as paints and varnishes, and in the manufacturing of coatings, adhesives, and sealants) can also trigger type II hypersensitivity responses.

**Type III** hypersensitivity responses (**immune complex-mediated hypersensitivity**) occur when antibodies, usually IgG or IgM, are formed against soluble antigens. The antigen–antibody complexes are deposited in tissues such as kidneys, joints, and lung vascular endothelium. These complexes activate leukocytes and complement within the tissue, which causes damage by initiating an inflammatory response. Type III hypersensitivity can be a result of the administration of **antivenins,** a condition called **serum sickness.** The antivenins, which are horse serum proteins obtained by inoculating a horse with the venom to be neutralized, function as antigens that elicit a type III response. Certain respiratory allergens, such as hay dust or mold spores in a farm, can also provoke a circulatory IgG response rather than an IgE response, resulting in a condition called **farmer's lung.**

A **type IV** hypersensitivity response (**delayed-type hypersensitivity**) is a result of activation of $T_H1$ and cytotoxic T cells. It most commonly presents as **contact dermatitis** when a toxin acts as a **hapten** and binds to host proteins. The first exposure does not normally produce a response, though subsequent dermal exposures can activate Langerhans cells, which migrate to the lymph nodes and activate T cells. The T cells then return to the skin and initiate an immune response. For example, the toxin in poison ivy, **urushiol,** causes its characteristic rash by acting as a hapten that binds epidermal cells. While contact dermatitis is caused by a reaction with extracellular proteins, certain chemicals, such as **pentadecacatechol,** are lipophilic, cross the cell cytoplasm and modify intracellular proteins. These modified proteins are then presented by MHC class I molecules and can be recognized by $CD8^+$ T cells. The cells cause cytotoxic damage directly and secrete cytokines eliciting an inflammatory response.

**Autoimmunity** results when the organism's immune system attacks its own cells (see Chapter 43, Pharmacology of Immunosuppression, for a discussion of autoimmunity). Several drugs and a number of other chemicals may cause autoimmune reactions. **Methyldopa** may cause a hemolytic anemia by eliciting an autoimmune reaction against the Rhesus antigens (Rh factors). Several other drugs, such as **hydralazine, isoniazid,** and **procainamide,** can cause a lupus-like syndrome by inducing antibodies to myeloperoxidase (for hydralazine and isoniazid) and DNA (for procainamide). The inhaled anesthetic **halothane** (see Chapter 15, General Anesthetic Pharmacology) can cause an autoimmune hepatitis in approximately 1 in 20,000 patients by altering a liver protein enough to elicit immune recognition. Chemicals such as **mercury** and **silica** can also cause autoimmune diseases. Chronic mercury exposure can induce the formation of anti-glomerular basement membrane antibodies resulting in a glomerular nephritis by inhibiting $CD8^+$ cells. Silica causes scleroderma by unknown mechanisms: properties of silica dust such as specific shape, crystalline structure, and size—along with airborne concentration—determine its toxic effects.

## Enzyme and Receptor-Mediated Toxicity

*Many compounds are acutely toxic because they alter important metabolic pathways or interact with critical receptors.* Interference with any metabolic or signaling pathway can cause toxicity, but commonly encountered toxins frequently interfere with neurotransmission, cardiac rhythm, oxygen delivery, ATP generation, or intracellular calcium homeostasis.

Some common toxins are mechanistically related to drugs that inhibit enzymes. For example, many common pesticides and **nerve gases** are acetylcholinesterase inhibitors (see Chapter 7) that are mechanistically analogous to **neostigmine** and **physostigmine.** Organophosphate nerve agents such as **tabun, sarin, soman,** and **VX** are lethal at much lower doses (as low as 10 mg for VX) than the carbamates used as therapeutics, because they form strong serine-phosphate bonds that irreversibly inhibit the enzyme. Thus, regaining cholinesterase function requires the de novo synthesis of acetylcholinesterase. Organophosphates have a highly reactive phosphate group that both mimics the tetrahedral intermediate in the acetylation reaction and forms a considerably stronger serine bond (Fig. 47-2). Moreover, the strength of the serine phosphate bond can be increased by "aging," whereby one of the alkyl phosphate bonds is hydrolyzed. In fact, nerve gases have been engineered to contain tertiary alkoxy groups that are particularly prone to hydrolysis, making these compounds especially toxic. Many pesticides, including **parathion, malathion, diazinon, fenthion, chlorpyrifos,** and **carbaryl,** are also acetylcholinesterase inhibitors, and with the exception of carbaryl, these agents are organophosphates. Because they were designed to show selectivity for arthropod cholinesterase, are preferentially toxified by arthropod enzymes, or are preferentially detoxified by mammalian carboxyesterases, organophosphate insecticides are considerably more toxic to arthropods than to humans, an example of **selective toxicity.**

The prototypical environmental toxin that acts by a receptor-mediated mechanism is **2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),** known commonly as **dioxin.** TCDD, to which people are exposed chiefly through consumption of contaminated fats in meat, fish and dairy products, binds to the **aryl hydrocarbon receptor** (**AhR**), dissociating it from a heat shock protein. This dissociation event allows the AhR to be phosphorylated by tyrosine kinase, activating the receptor. Activated AhR is a nuclear protein that enhances the transcription of genes encoding the CYP1A family of cytochrome P450 proteins. The affinity of the AhR for TCDD varies greatly (>1,000 fold) among species, helping to explain the greatly different sensitivities of different animals to the adverse actions of TCDD. TCDD was a contaminant of **Agent Orange,** the defoliant used extensively in the Vietnam War.

Agents that inactivate hemoglobin can inhibit oxygen delivery. **Carbon monoxide** binds strongly to the iron in the active site of hemoglobin, preventing oxygen binding and reducing oxygen carrying capacity. In addition, carbon monoxide increases hemoglobin's affinity for oxygen at the other oxygen binding sites (see Fig. 47-3). Although this might seem to be beneficial, the increased binding affinity prevents unloading of oxygen at the tissues, reducing oxygen delivery. A number of other substances (including **trinitrotoluene, amyl nitrate, metochlopramide,** and **primaquine**) can oxidize the heme iron in hemoglobin to its $Fe^{3+}$ state, forming **methemoglobin.** Methemoglobin is unable to bind to oxygen, and like carbon monoxide, can lead to tissue hypoxia and death.

**Cyanide,** as gaseous hydrogen cyanide or its soluble salts, interferes with cellular energy production. Cyanide binds to the heme iron in the active site of the enzyme **cytochrome *c* oxidase,** which is responsible for the final step of the electron transport chain, the reduction of oxygen to water, in mito-

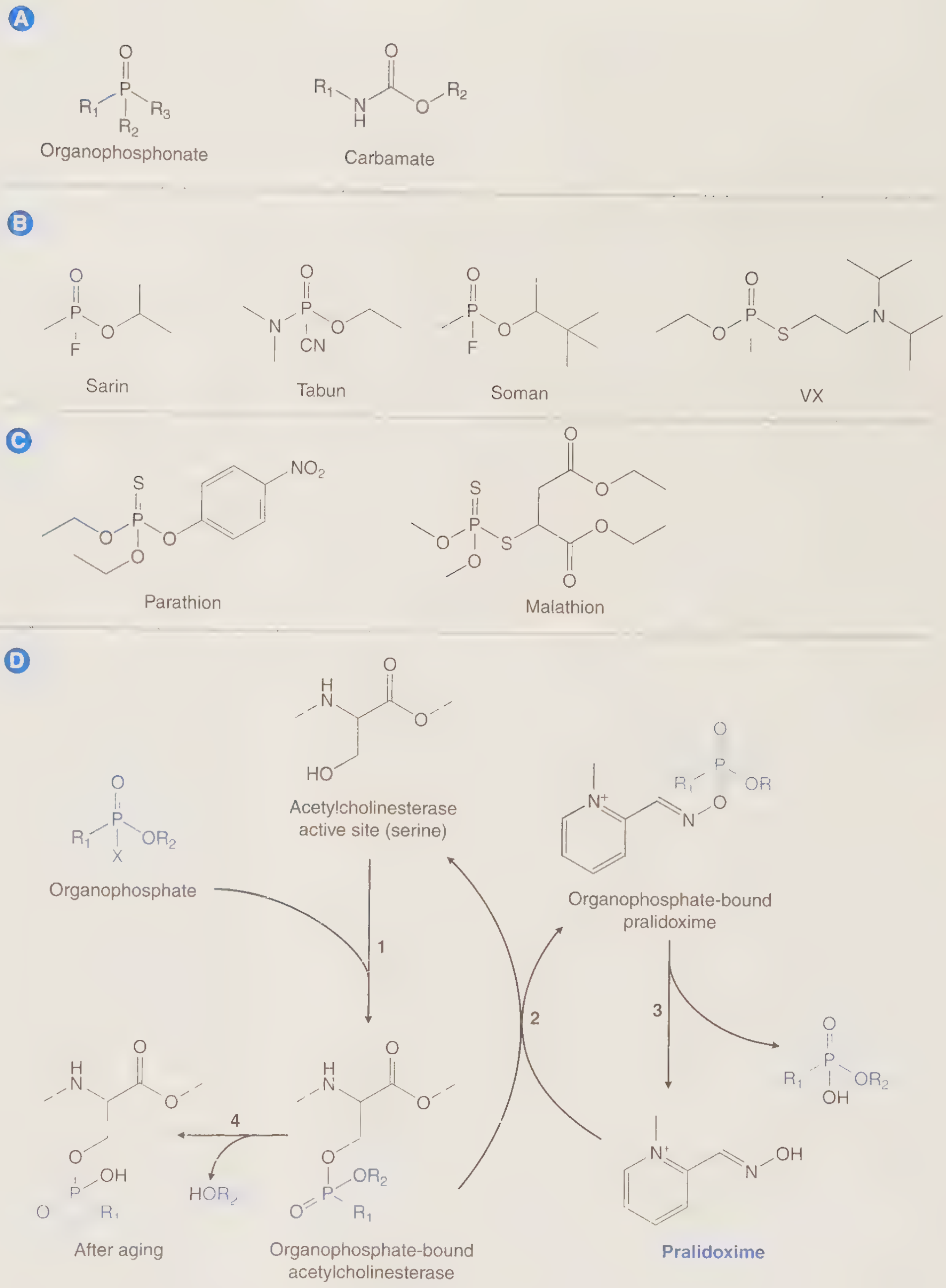

A
Organophosphonate
Carbamate
B
Sarin
Tabun
Soman
VX
C
Parathion
Malathion
D
Organophosphate
Acetylcholinesterase active site (serine)
Organophosphate-bound pralidoxime
1
2
3
4
HOR₂
After aging
Organophosphate-bound acetylcholinesterase
Pralidoxime

**Figure 47-2. Structures and Mechanisms of Acetylcholinesterase Inhibitors.** **A.** Structures of typical acetylcholinesterase inhibitors, an organophosphonate on the left and a carbamate on the right. **B.** Structures of the principal nerve gases sarin, tabun, soman, and VX. **C.** Structures of the organophosphate insecticides parathion and malathion. The thiophosphate bonds between the sulfur and phosphorus are oxidized more efficiently by arthropod oxygenases than by mammalian oxygenases, so the compounds are less toxic to humans than the structurally related nerve gases. **D.** Organophosphates attack the serine active site in acetylcholinesterase, forming a stable phosphorus-oxygen species (1). Pralidoxime abstracts the organophosphate from serine, restoring active acetylcholinesterase (2). Organophosphate-bound pralidoxime is unstable and spontaneously regenerates pralidoxime (3). Organophosphate-bound acetylcholinesterase can lose an alkoxy group, in a process called aging. The end-product of aging is much more stable and cannot be detoxified by pralidoxime.

chondria. Inhibition of this enzyme results in an accumulation of electrons in the electron transport chain and prevents the generation of ATP.

## CELLULAR REPAIR MECHANISMS AND CELL DEATH

Because cells have repair mechanisms, toxic exposure leading to cellular dysfunction does not necessarily lead to cell death. Damaged macromolecules can be repaired by a number of different mechanisms. For example, oxidized thiol groups on proteins can be reduced by thioredoxin and glutaredoxin. Denatured proteins can be refolded by molecular chaperones, such as heat shock proteins, which can be produced in response to cellular injury. Those proteins that cannot be repaired can be degraded by the proteolytic action of lysosomal proteases or the ATP/ubiquitin-dependent system and replaced. Likewise, oxidized lipids can be reduced or hydrolyzed and replaced.

The most important macromolecular repair mechanisms are those involved in the repair of DNA. In part because of the double-stranded structure of DNA, it is also the macromolecule most amenable to repair, because damaged nucleic acids can be excised and replaced through a complex excision-repair mechanism using the sister strand as a template. DNA repair can also be accomplished by direct repair of covalent modifications (such as alkylation). In some cases, DNA can be repaired *after* replication, by recombination (crossover) of the damaged daughter strand with its healthy sibling.

Failure of cellular repair can lead to cell death. Depending on the severity of toxicity, a cell may undergo **apoptosis** (programmed cell death). Apoptosis allows the cell to undergo

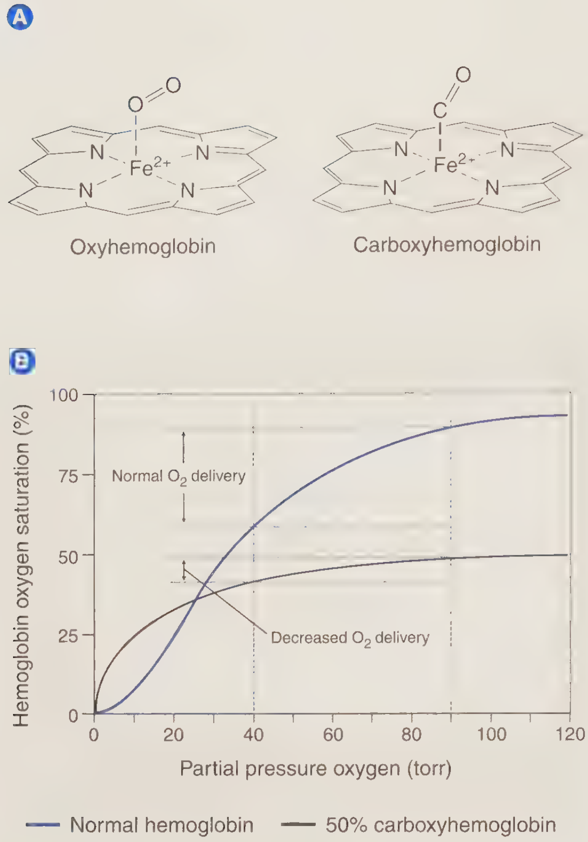


**Figure 47-3. Mechanism of Carbon Monoxide Poisoning.** **A.** The oxygen binding site of hemoglobin is a ferrous heme that can reversibly bind oxygen. Carbon monoxide prevents oxygen binding by forming a bond to ferrous heme that is significantly stronger than the heme-oxygen bond (indicated by a shorter bold line). **B.** Carbon monoxide interferes strongly with oxygen transport not only because it prevents oxygen binding but also because it increases the affinity of heme for oxygen. Under normal conditions (blue line), hemoglobin is 85% saturated with oxygen in the alveoli (where the partial pressure of oxygen is approximately 90 torr). At tissue partial pressures (40 torr), normal hemoglobin is 60% saturated with $O_2$. Thus, under normal conditions, 25% of the hemoglobin sites deliver their oxygen to the tissues. When 50% of oxygen binding sites are occupied by carbon monoxide (black line), hemoglobin oxygen saturation can be no more than 50% at a partial pressure of 90 torr. At tissue partial pressures (40 torr), the hemoglobin oxygen saturation is still over 35%, indicating that less than 15% of the heme sites have delivered their oxygen to the tissues.

a relatively ordered and coordinated destruction by activation of a number of dedicated proteins. Apoptosis results in cell shrinkage, nuclear condensation, and the formation of **apoptotic bodies,** membrane-bound structures composed of cytoplasm and tightly bound organelles that can be phagocytosed by macrophages or healthy neighboring tissue. If the toxicity is significant enough that an ordered cell death cannot be accomplished, usually because there is insufficient cellular energy to carry out the process, the cell undergoes **necrosis.** Necrosis is characterized by enzymatic digestion of cellular contents, denaturation of cellular proteins, and disruption of cellular membranes. Unlike apoptotic cells, necrotic cells are swollen and do not form apoptotic bodies. While apoptotic cells undergo cell death with minimal inflammation and disruption to adjacent tissue, necrotic cells attract inflammatory cells and can damage nearby healthy cells. For this reason, apoptosis causes milder tissue and organ dysfunction and is preferable to necrosis as a means to eliminate damaged cells. Certain mutations in cancer cells prevent apoptosis and, therefore, favor proliferation of the malignant cells.

## ORGAN-DIRECTED TOXICITY

The pattern of toxicity observed following cellular damage is largely determined by the regenerative capacity of the target organ system. In those organs that are capable of regeneration, such as the liver, repeated insults resulting in tissue necrosis may be followed by regeneration of the lost tissue. Over time, however, cellular injury can lead to excessive deposition of collagen and extracellular matrix proteins, causing **fibrosis.** Organ systems with limited or no regenerative function, such as cardiac and neuronal tissue, lose function as tissue is destroyed from toxic exposure. A third pattern of organ-specific toxicity, the neoplastic transformation of cells, is described below (see Carcinogenesis).

In the liver, hepatic toxicity can follow three principal patterns: hepatic necrosis, hepatic inflammation (**hepatitis**), and cholestasis. Hepatic necrosis usually follows a regional pattern, and many toxins, including acetaminophen and carbon tetrachloride, affect primarily the region of the liver near the hepatic vein, a pattern termed **centrilobular necrosis.** Hepatitis is caused by an inflammatory reaction. Although most frequently associated with infectious hepatitis, an autoimmune hepatitis can be triggered, for example, by halothane, as mentioned above. Cholestasis results from the accumulation of bile in the canaliculi and can result from biliary duct obstruction or hepatocellular dysfunction. It is commonly associated with exposure to **organic arsenic compounds,** with **oral contraceptives,** and **anabolic steroids.**

The liver is capable of almost complete regeneration after most toxic exposures. Chronic liver toxicity is manifest principally as hepatic **cirrhosis,** most commonly seen secondary to chronic **ethanol** abuse. However, virtually all hepatotoxic compounds can lead to cirrhosis.

Chronic toxicity in the lungs can be manifested as both loss of function and fibrosis. Emphysematous changes caused by loss of gas-exchange membranes are caused by the destruction of pulmonary **elastin** by neutrophil-derived **elastase.** Thus, toxins that elicit an inflammatory response, such as those found in **cigarette smoke,** can lead to **emphysema. Pulmonary fibrosis,** on the other hand, is caused by excessive and abnormal collagen deposition in the alveolar interstitium and the centriacinar region. Numerous occupational exposures can induce pulmonary fibrosis, including materials such as **asbestos, beryllium, coal dust, silica,** and **talc,** and chemicals such as **ammonia, chlorine, isocyanates, phosgene, ozone,** and even high concentrations of **oxygen.**

Because neurons are incapable of regeneration, neuronal loss caused by toxic exposures is essentially permanent. Fortunately, the **blood–brain barrier** (discussed in Chapter 6) excludes many toxic compounds from the central nervous system. Nonetheless, nonpolar toxins, in particular, are able to cross the blood–brain barrier. In the peripheral nervous system, neurotoxins frequently affect Schwann cells, causing a peripheral neuropathy. Because these cells are capable of regeneration, the neuropathy is usually at least partially reversible.

Widespread neuronal loss leads to diffuse encephalopathy, as in the case of **methyl mercury.** Many neurotoxins affect specific types of neurons. For example, the compound **1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTP),** an accidental contaminant of illegally synthesized **meperidine,** is selectively toxic to dopaminergic neurons in the substantia nigra, and consequently leads to Parkinsonism (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission). **Formic acid,** a metabolite of **methanol,** is a neurotoxin that causes retinal and optic nerve damage. Although formic acid is incapable of crossing the blood–brain barrier, retinal cells contain the enzymes necessary to convert methanol to formic acid, so ingestion of methanol leads to neuronal destruction and subsequent blindness.

Chronic renal failure occurs because loss of sufficient glomeruli diminishes the glomerular filtration rate. The kidney is particularly susceptible to toxins because it concentrates many xenobiotics for excretion. Nephrotoxicity results from a variety of mechanisms, including the alteration of renal hemodynamics, tubular damage and obstruction, glomerular nephropathy, and interstitial nephritis. Although the kidney is able to recover from many acute toxic insults that do not destroy glomeruli, loss of some kidney function can result in adaptive compensation by functioning glomeruli that can maintain the glomerular filtration rate. When sufficient nephrons are lost, compensatory hemodynamic changes increase glomerular pressures, leading to glomerular sclerosis, further glomerular loss and progressive renal failure. Aside from the multitude of drugs that can cause renal failure, including many **antibiotics, NSAIDs,** and **angiotensin converting enzyme inhibitors,** a number of xenobiotics are particularly nephrotoxic. Many **heavy metals,** particularly inorganic salts of mercury and **cadmium,** cause nephrotoxicity, as do some halogenated hydrocarbons and oxalic acid, a metabolite of ethylene glycol (antifreeze).

## CARCINOGENESIS

**Carcinogenesis** is the transformation of a normal cell to a neoplastic cell, followed by a clonal expansion. A **carcinogen** is a chemical, physical, or biological substance that can cause transformation. Carcinogenesis is a complex process that usually requires years to decades to complete in human beings, and involves multiple genetic changes, only some of which can be attributed to carcinogens. As a result, cancer incidence tends to increase exponentially with age, except for certain cancers of childhood. Moreover, although some 90 human carcinogenic agents have been identified so far and more are suspected, many important types of malignancies, such as cancers of the colon, breast, and prostate, have no established exogenous causes.

The development of cancer requires sequential genetic changes (the first of which is termed **initiation**) and epigenetic changes (characterized as **promotion** and **progression**). Molecularly, **initiators** act by damaging DNA, interfering with DNA replication, or interfering with DNA repair mechanisms. Most initiators are reactive species that chemically modify the structure of DNA, preventing accurate replication and leading to a mutation. For example, planar chemicals such as **polycyclic aromatic hydrocarbons** and their metabolites can intercalate between base pairs of DNA, causing frameshift mutations. Others, such as the **nitrogen mustards,** alkylate DNA and induce point mutations. Like most reactive species, initiators are frequently toxified by the metabolic action of cytochrome P450 and other enzymes. The **precarcinogens** are activated by phase I reactions into the **ultimate carcinogen,** which is the form that actually interacts with DNA and causes the mutations that lead to neoplastic transformation. For example, **benzo[a]pyrene** is a precarcinogen planar molecule composed of five fused aromatic rings that, when oxidized in a specific region, termed the "bay region," forms a dihydroxy epoxide metabolite that can cause double-stranded breaks in DNA (see Fig. 47-4). Oxidation of a different region, termed the K region, enables conjugation with glucuronate or glutathione, allowing the compound to be eliminated.

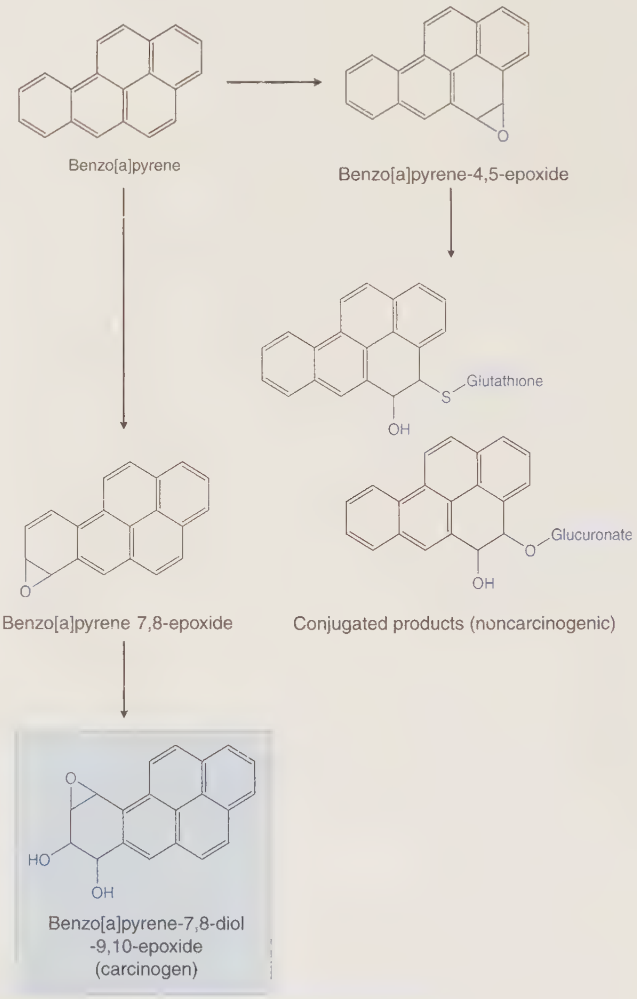

Benzo[a]pyrene
Benzo[a]pyrene-4,5-epoxide
O
Glutathione
S
OH
Glucuronate
O
OH
Conjugated products (noncarcinogenic)
O
Benzo[a]pyrene 7,8-epoxide
O
HO
OH
Benzo[a]pyrene-7,8-diol -9,10-epoxide (carcinogen)

**Figure 47-4. Metabolism of Benzo[a]pyrene.** Benzo[a]pyrene is a precarcinogen that can be metabolized by a variety of pathways. Oxidation at the so-called bay region produces the ultimate carcinogen benzo[a]pyrene-7,8-diol-9,10-epoxide, which can cause double-stranded breaks in DNA. On the other hand, oxidation at the so-called K region produces the 4,5-epoxide of benzo[a]pyrene. Opening of the epoxide and conjugation with glutathione or glucuronate produces noncarcinogenic conjugated products that can be excreted.

In most cases, DNA mutations are repaired, are silent, or are lethal to the cell. However, in a small number of cases, the DNA mutations are passed on during cell replication. If those mutations affect genes that control cell cycle regulation, neoplastic transformation may be initiated.

*Carcinogenesis typically involves mutation in at least two types of genes.* **Proto-oncogenes** encode proteins that encourage cell-cycle progression. These gene products include growth factors, growth factor receptors, certain signal transduction pathway proteins, cell cycle kinases, and transcription factors. Mutations of proto-oncogenes that result in constitutive activation or overexpression, such as mutations of promoter regions, can initiate neoplastic transformation.

**Tumor suppressor genes** encode proteins responsible for the inhibition of cell-cycle progression. These inhibitors inactivate signal transduction pathways that encourage cell growth. Inhibitors of cell-cycle progression include the cyclin-dependent protein kinase inhibitors, transcription factors that stimulate the transcription of other tumor suppressors, or transcription factor inhibitors that block the transcription of proto-oncogenes. Mutations that inactivate tumor suppressor genes, such as missense mutations, may lead to neoplastic transformation.

One of the most important tumor suppressor gene products is a 53-kilodalton protein, called p53. Normally functioning p53 is involved in regulating cell division, repairing DNA, and initiating **apoptosis** (programmed cell death). Individuals with inherited mutations in the p53 gene, such as those with Li-Fraumeni syndrome, are unusually likely to develop childhood sarcomas, early breast cancer, or other types of cancer. Acquired point mutations in this gene are common in many forms of cancer: for example, p53 mutations tend to be more prevalent in smokers than nonsmokers.

**Tumor promoters** alter the cell cycle either to encourage cell growth and division or to prevent apoptosis. Some promoters act as **mitogens,** sending extracellular signals that encourage mitosis, or stimulate signal transduction pathways involved in cell cycle regulation. For example, certain exogenous hormones (such as estrogen) or synthetic hormone analogues can lead to the development of tumors in their target organs. **Phenobarbital** is a promoter used extensively in experimental cancer research. Carcinogens can also promote the development of cancer by causing chronic tissue damage in organs that are capable of regeneration, such as the liver. For example, chronic consumption of high levels of ethanol can lead to cirrhosis, which can lead to hepatocellular cancer, believed to be the result of chronic tissue regeneration and repair.

## TERATOGENESIS

**Teratogenesis** is the creation of birth defects, and a **teratogen** is a substance that can induce such birth defects. In 1959, James Wilson published a monograph detailing six basic principles of teratology that remain the cornerstone of developmental toxicology. They are:

- Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which this interacts with adverse environmental factors.
- Susceptibility to teratogenesis varies with the developmental stage of the fetus at the time of exposure to an adverse influence.
- Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis).
- The access of adverse agents to developing tissues depends on the nature of the agent.
- The four manifestations of deviant development are death, malformation, growth retardation, and functional deficit.
- Manifestations of deviant development increase in frequency and degree as dosage increases, from the no-effect to the totally lethal level.

Because organ system development is precisely timed, *the teratogenic effect of any substance is dependent on the developmental timing of the exposure.* For example, **retinoic acid** (vitamin A) is a potent teratogen that binds to and activates nuclear retinoid receptors that regulate a number of key transcriptional events during development. When given to hamsters between the 7th and 9th days of gestation (when the tail is formed), retinoic acid results in a short or absent tail. If exposure occurs between the 9th and 10th days of gestation, however, retinoic acid causes shortened forelimbs and cleft palates. In humans, **organogenesis,** the development of most recognizable body structures, occurs between the 3rd and 8th weeks of gestation (one exception is development of the uretheral groove in males, which occurs as late as the 90th day of gestation). It is during the period of organogenesis that teratogens are most potent. Before the third week, most toxic compounds result in death to the embryo and spontaneous abortion, whereas after organogenesis, teratogenic compounds may affect growth and functional maturation of organs, but do not affect the most basic developmental plan.

Like other toxins, teratogens are active at the molecular level. Most teratogens are reactive species or metabolic inhibitors. In particular, substances that can inhibit intercellular signals, such as growth factors and hormones, or their downstream intracellular messengers and transcription factors, are frequently potent teratogens. Teratogens can also cause birth defects by altering DNA or chromosomes, by affecting cellular energy production or availability, by affecting the supply of metabolic precursors needed for cell growth, by interfering with the cell cycle, or by otherwise interfering with cellular proliferation, division, differentiation, movement, apoptosis, or cell–cell interactions in the developing embryo.

Exposure of the fetus to a teratogen necessarily involves maternal exposure. For this reason, the interaction between maternal tissues and the toxin is important to fetal exposures. In particular, the fetus's exposure to the agent is determined by maternal toxicokinetics, metabolism, and clearance, by the toxification of inert precursors in maternal tissues, and by the ability of the teratogen to cross the maternal–fetal barrier through the placenta.

Low-level teratogenic exposures on an individual level are believed to follow a threshold pattern of dose response. In other words, for each individual, there is a level of teratogenic exposure below which maternal defense mechanisms and embryonic repair mechanisms are capable of preventing teratogenesis. Above the threshold level, there is likely a continuum between growth retardation, increasingly severe birth defects, and death.

## PREVALENT TOXIC EXPOSURES

### CIGARETTE SMOKE

**Cigarette smoke** is the most commonly encountered and important toxin in America, responsible for about 30% of all U.S. cancer deaths and significantly increased risks of pulmonary disease and cardiovascular disease. Tobacco smoke causes not only lung cancer but also cancers of the oral cavity, esophagus, pancreas, and bladder. Passive smoking—that is, the exposure of nonsmokers to cigarette side-stream smoke—is also believed to cause cancer and cardiovascular disease, although the magnitude of these risks is less clear than it is for smokers. The carcinogenicity of cigarette smoke is likely due to the combined actions of many of the dozens of carcinogens among the 4,000 chemicals in cigarette smoke. Several of these carcinogens are not limited to the particle phase, or "tar," but are in the gas phase as well. "Low tar" cigarettes are as carcinogenic and cause as much cardiovascular disease as "regular" cigarettes.

### ETHANOL

Excessive consumption of **ethyl alcohol** is also a common and complex toxic exposure. Binge drinking occurs in a sizable minority of adolescents, and in adults with coronary artery disease, binge drinking can cause myocardial ischemia and angina. Acutely, alcohol is a sedative (see Chapter 10, Pharmacology of Excitatory and Inhibitory Neurotransmission and Chapter 16, Pharmacology of Chemical Dependence and Addiction) and causes psychomotor retardation. The largest fraction of morbidity and mortality from alcohol intoxication results from injuries suffered (not to mention inflicted) while impaired.

Chronic excess drinking leads to increased risk of liver cirrhosis, hepatocellular carcinoma, pancreatitis, hemorrhagic stroke, and congestive heart failure. The pathophysiology of the last of these diseases, alcoholic cardiomyopathy, is complex and seems to involve cell death and various adverse changes in myocyte function. Women tend to be more susceptible than men to alcoholic cardiomyopathy.

The mechanism of alcoholic hepatotoxicity is also multifactorial. First, it is associated with a nutritional deficiency, as chronic alcohol abusers obtain most of their calories from alcohol. This leads to a hypermetabolic state and increased oxygen demand in the liver. In turn, poorly perfused centrilobular hepatocytes are threatened. The metabolism of alcohol generates considerable NADH and NADPH, which shift the redox potential of the hepatocyte. The altered redox potential leads to increased production of lactic acid and uric acid and to hyperglycemia. Finally, ethanol metabolism produces reactive species, including acetaldehyde (produced by the action of alcohol dehydrogenase) and hydroxyl radicals, superoxide anions, and hydrogen peroxide (produced by the action of the P450 enzyme CYP2E1).

Ethanol is believed to cause tumors in the oropharynx, larynx, and esophagus directly, where it may act synergistically with tobacco smoke. Ethanol is also associated with hepatocellular carcinoma, both by inducing chronic regeneration of damaged tissue (see above), and by inducing CYP2E1, which can activate carcinogens.

Ethanol is also a teratogen, and causes **fetal alcohol syndrome,** the most common preventable cause of mental retardation. Despite significant research, the mechanism of fetal alcohol syndrome remains unknown.

Light-to-moderate ingestion of alcohol, on the other hand, appears to protect against heart disease. The mechanisms that modulate this risk reduction may include increased production of high-density lipoprotein cholesterol, plasminogen and tissue plasminogen activator, coupled with decreased platelet aggregation, fibrinogen, and lipoprotein(a) and with altered endothelial function.

### FOOD POISONING

**Food poisoning** is also a prevalent toxicologic problem: an estimated one in four Americans experience significant foodborne illnesses each year. The mechanisms of food poisoning involve either infection, which typically manifests one to several days after exposure, or intoxication from a preformed microbial or algal toxin, with symptoms occurring within hours following exposure. Infectious food poisoning is typically caused by species of *Salmonella, Listeria, Cryptosporidium,* or *Campylobacter.* Less common, but quite virulent, are poisonings by enteropathogenic *E. coli,* which can cause sometimes fatal hemorrhagic colitis and hemolytic-uremic syndrome (HUS), likely through the uptake of pathologic bacterial proteins by host cells.

Food intoxication is often caused by toxins elaborated by *Staphylococcus aureus* or *Bacillus cereus,* or by marine algal toxins ingested via seafood. *S. aureus* produces a variety of toxins, of which the Staphylococcal enterotoxins (SE) cause emesis by stimulating receptors in the abdominal viscera. Food handling after cooking, followed by poor refrigeration, contaminates high-protein foods such as meats, cold cuts, and egg and dairy products.

*B. cereus* is a common contaminant of cooked rice. It produces several toxins that cause vomiting and diarrhea in exposed individuals. Of particular concern is the production of **cerulide,** a small, cyclic peptide that stimulates intestinal 5-HT$_3$ receptors, resulting in emesis. The peptide is heat-stable to 259°F for up to 90 minutes, so cooking does not necessarily prevent intoxication.

Most algal toxins are neurotoxic and heat-stable, so that cooking leaves the toxins intact. The algal toxins such as **saxitoxins,** for example, are a group of approximately 20 heterocyclic guanidines that bind with high affinity to the voltage-dependent sodium channel, thus inhibiting neuronal activity and causing tingling and numbness, loss of motor control, drowsiness, incoherence, and, with sufficient doses (greater than about 1 milligram), respiratory paralysis.

Many foodborne illnesses seem to be caused by pathogens not yet characterized. It is believed that novel pathogens can emerge because of changing ecologies or technologies, or can arise via transfer of mobile virulence factors, such as bacteriophage.

### LEAD

Lead is ubiquitous in the environment, both because of its persistence and because of its formerly widespread use in paints, plumbing, solder, and as an additive in gasoline. Lead exposure causes neural toxicity, making exposure a particular concern for fetuses and children up to the age of about 7 years. Young children are also at risk because they are more likely than adults to eat lead-contaminated paint dust or soil. Although the half-life of lead in soft tissues is relatively short, its half-life in bone is more than 20 years; a substantial exposure in early childhood could result in elevated lead levels for decades. Despite a five-fold decrease in the exposure to lead in the United States since the 1940s, nearly one million U.S. children are believed to be at risk for lead poisoning. Some minority and impoverished children are at particular risk because of a combination of residence in improperly maintained, formerly lead-painted dwellings, waterborne exposures from old plumbing, and/or inadequate dietary intakes of calcium and iron.

Lead causes a disruption of the blood–brain barrier, allowing both lead and other potential neurotoxins to reach the central nervous system. There, lead can block voltage-dependent calcium channels, interfere with neurotransmitter function, and, most importantly, interfere with cell–cell interactions in the brain, which causes permanent changes in the neuronal circuitry. Overt lead encephalopathy, which is rare in the U.S. today, results in lethargy, vomiting, irritability, and dizziness and can progress to altered mental status, coma, and death. More important is the risk, in children, of an IQ deficit of approximately two to four points for every ten microgram-per-deciliter increase in blood lead concentration.

Lead interferes with the synthesis of hemoglobin at multiple steps, causing a microcytic, hypochromic anemia. In particular, lead inhibits the action of d-aminolevulonic acid dehydratase (ALA-D), which synthesizes porphobilinogen, a precursor to the heme group. Lead also inhibits the incorporation of iron into the porphyrin ring.

In the kidney, lead causes both reversible and irreversible toxicity. Lead can interfere reversibly with the energy production of proximal tubular cells by interfering with mitochondrial function, resulting in decreased energy-dependent reabsorption of ions, glucose, and amino acids. Chronic exposure to lead results in interstitial nephritis, with the development of interstitial fibrosis and chronic renal insufficiency.

## TREATMENT FOR ACUTE EXPOSURES

Several general therapeutic principles apply to all toxic exposures. The principles of treating any exposed patient are to remove the patient from the contaminated environment, to stabilize the patient with standard life-supportive measures, and to identify the toxic exposure.

Once a poison has been identified, several general strategies are used to minimize harm, based on the nature of the inciting toxin. One strategy is to alter the toxicokinetics of the poison so as to minimize exposure by decreasing absorption, preventing toxication of a benign substance, or increasing the metabolism or elimination of a toxin. A second strategy aims to inactivate a toxin by binding a small molecule or antibody to it, thus preventing the toxin from interfering with specific biochemical and cellular processes. A final strategy is to counteract a toxin's effect at the biochemical, cellular, or whole-body level.

Table 47-1 lists the major poisons, including mechanism, receptor or target, clinical signs, and antidote, if any.

## PRINCIPLES FOR TREATING THE ACUTELY POISONED PATIENT

The most important principle for treating a poisoned patient is to remove the exposure. There is no effective treatment in the face of continued exposure to a toxic substance; limiting the exposure is, therefore, imperative. In many cases, this step alone is effective because the body's homeostatic mechanisms (discussed above) can respond adequately to some brief exposures to minimize injury. For example, acute symptoms caused by inhalation of high concentrations of **trichloroethylene** vapor are readily reversed after removal of the patient to fresh air.

Once the patient has been removed from the exposure, standard supportive measures such as securing the airway, ensuring ventilation and end-organ perfusion, and correcting electrolyte abnormalities are undertaken. For example, overdose of a **β-adrenergic antagonist** is treated by giving parenteral fluids aggressively to treat hypotension, and, if necessary, pacing the heart with external paddles or atrial pacing to combat bradycardia. Likewise, **salicylate** poisoning, which causes a metabolic acidosis, is treated with aggressive electrolyte management, including the administration of **sodium bicarbonate** to alkalinize the blood.

The next step is to establish the nature of the poison. Sometimes this is known from the history, such as an occupational exposure, or from evidence gathered at the scene, such as an empty pill bottle. Other times, the patient's symptoms are consistent with a particular **toxidrome.** For example, the symptoms of cholinergic overload (vomiting, diarrhea, peripheral vasodilation, loss of accommodation, and pupillary dilatation) in an agricultural setting might suggest exposure to a pesticide. Frequently, however, particularly in an obtunded patient, it may be necessary to obtain plasma toxicology data or other specialized information to determine definitively the poison and to tailor treatment accordingly.

## TOXICOKINETIC-BASED TREATMENTS

Toxicokinetic approaches aim to minimize the opportunity for the poison to cause end-organ damage by decreasing the quantity of the drug in the body. This can be accomplished by preventing the poison from being absorbed, preventing toxication, enhancing the toxin's metabolism, or by enhancing its elimination.

### Prevention of Absorption

As stated earlier, *the best way to prevent further absorption of a toxic compound is to eliminate the exposure.* In addition, it may be possible to prevent gastrointestinal absorption of material that has been ingested but not yet absorbed by inducing emesis or performing a gastric lavage through a nasogastric tube in cases where the poison is still in the stomach (within 1 hour of ingestion). The most common method of inducing emesis is with **ipecac syrup,** which acts via a dual mechanism

*(text continues on page 738)*

**TABLE 47-1** Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Acetaminophen | Converted to reactive NAPQI in the liver | N/A | Hepatotoxicity | N-acetylcysteine |
| Acid anhydrides | Strong nucleophiles; cause type II hypersensitivity reactions | N/A | Eye and skin irritants | None |
| Amyl nitrite | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Anabolic steroids | | Steroid receptors | Lipoprotein disturbances, cardiac disease, hepatoma, psychiatric disturbances, and hypogonadal states | None |
| Aniline dyes | Oxidize hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Antivenins | Cause type III hypersensitivity reactions | N/A | Serum sickness | None |
| Arsenic | May replace phosphate in several reactions; binds cysteine residues in proteins; alters DNA methylation; oxidative stress; alters cell proliferation; tumor promoter | N/A | Carcinogen (skin, lung, liver) | Dimercaprol |
| Asbestos | Inhaled fibers lodged in alveoli. Fibers elicit iron deposition. Fibers also attract macrophages → inflammatory reaction → collagen formation | N/A | Pulmonary fibrosis, lung cancer, mesothelioma, maybe gastrointestinal cancer | None |
| Atropine | Antagonist at cholinergic receptors | Muscarinic cholinergic receptors | Glaucoma, tachycardia | Physostigmine |
| Benzene | Converted to reactive phenols and polyphenols in the liver | N/A | CNS depression (acutely)<br>Bone marrow depression, leukemia (chronically) | None |

*(continued)*

**TABLE 47-1** Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Benzocaine | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Beryllium | Inhaled particles can elicit granuloma formation | N/A | Chemical pneumonitis; suspected carcinogen; lung hypersensitivity | None |
| Botulinum toxin | Blocks release of acetylcholine | Synaptobrevin or synapse-associated proteins | Muscular paralysis | None |
| Brodifacoum | Prevents clotting factor synthesis by inhibiting vitamin K regeneration | Epoxide reductase | Bleeding | Vitamin K<br>Fresh frozen plasma |
| Cadmium | Inhibits synthesis of alpha-1-antitrypsin; causes proteinuria | N/A | Carcinogenic; vomiting, pulmonary edema (acute); emphysema, renal failure, osteomalacia (chronic) | Succimer |
| Calcium hydroxide | Caustic (alkali) agent; chemical burns | N/A | Chemical burns | None |
| Carbon monoxide | Binds to hemoglobin, preventing oxygen transport; shifts oxygen saturation curve to the left, preventing oxygen unloading | Hemoglobin | Headache, cardiac ischemia (in patients with pre-existing heart disease), lactic acidosis; neurologic symptoms including seizures, coma, and death | Hyperbaric oxygen |
| Carbon tetrachloride | Converted to reactive trichloro radical in the liver | N/A | Hepatotoxicity, renal toxicity | None |
| Chlorpyrifos | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Cigarette smoke |  | N/A | Lung cancer, oropharyngeal cancer, other cancers; emphysema; cardiovascular disease | None |

*(continued)*

**TABLE 47-1** Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Coal dust | Fine particles irritate the lungs and mucous membranes | N/A | Mucous membrane irritation; pulmonary irritation; pneumoconiosis | None |
| Copper | Generates free radicals | N/A | Hepatotoxicity; cerebellar dysfunction; hematuria, proteinuria, oliguria, and/or uremia | Penicillamine, trientine |
| Cyanide | Binds to cytochrome *c* oxidase, preventing reduction of oxygen to water, halting electron transport chain | Cytochrome *c* oxidase | Lactic acidosis, irregular respirations; convulsions, coma; death | Amyl nitrite<br>Sodium nitrite<br>Sodium thiosulfate |
| Diazinon | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Digoxin | Increases intracellular $Ca^{2+}$ concentration | $Na^+/K^+$ ATPase | Ventricular extrasystole, AV block | Digoxin Fab |
| Diisocyanates | Cause type II hypersensitivity reactions | N/A | Atypical asthma; respiratory irritation | None |
| Dioxin | Various; induces P450 via Ah receptor | Aryl hydrocarbon receptor | Possible carcinogenesis; porphyria cutanea tarda; chloracne | None |
| Diphacinone | Prevents clotting factor synthesis by inhibiting vitamin K regeneration | Epoxide reductase | Bleeding | Vitamin K<br>Fresh frozen plasma |
| Ethanol | Stimulates GABA receptors, inhibits glutamate receptors; nutritional deficiencies; metabolized to toxic intermediates (acetaldehyde) | GABAergic receptors (enhances effect), glutamatergic receptors (inhibitor) | CNS depression (acutely); hepatic cirrhosis, cardiomyopathy (chronic); fetal alcohol syndrome | None |
| Ethylene glycol | Converted to oxalic acid → calcium oxalate crystals in the kidney | N/A | Acute renal failure | Ethanol<br>Fomepizole |

*(continued)*

**TABLE 47-1** Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Fenthion | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Glycol ethers | Direct nephrotoxin | N/A | Kidney and liver damage | None |
| Halothane | Alters liver proteins to elicit autoimmune reaction | N/A | Autoimmune hepatitis | None |
| Hay dust | Causes type I, type III hypersensitivity reaction | N/A | Allergic rhinitis; asthmatic bronchoconstriction; hypersensitivity pneumonitis | Antihistamines<br>Glucocorticoids |
| Hydralazine | Causes autoimmune reaction by inducing autoantibodies against myeloperoxidase | N/A | Lupus-like syndrome | None |
| Hydrochloric acid | Strong acid; chemical burns | N/A | Chemical burns | None |
| Hydrofluoric acid | Strong acid; leaches bone → hypercalcemia | N/A | Chemical burns, hypercalcemia | Treatment for hypercalcemia |
| Iron | Generates free radicals; accumulates in cardiac tissue with hemosiderin → cellular degeneration and fibrosis | N/A | Hepatotoxicity, cardiotoxicity | Deferoxamine |
| Isoniazid | Causes autoimmune reaction by inducing autoantibodies against myeloperoxidase | N/A | Lupus-like syndrome | None |
| Lead | Binds to cysteine residues in proteins. Destroys blood–brain barrier; interferes with voltage-dependent calcium channels, neuronal cell–cell signaling, and neurotransmitter function; inhibits hemoglobin synthesis | Multiple: Pyrimidine 5′-nucleotidase; δ-aminolevulonic acid dehydratase (ALA-δ) | Hypochromic anemia; developmental delays; encephalopathy; probable human carcinogen; renal toxicity; peripheral neuropathy | Calcium disodium edetate<br>Dimercaprol<br>Succimer |

*(continued)*

## TABLE 47-1 Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Malathion | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Mercury | Alkyl compounds are direct neurotoxins (reactive species); mercuric salts bind cysteine residues in proteins, causing changes in protein structure | N/A | Interstitial pneumonitis, membranous colitis (acute); psychotic reactions, renal failure, muscle tremors, dementia, cerebral palsy, mental retardation (chronic); teratogen | Dimercaprol<br>Succimer |
| Methanol | Liver: formaldehyde<br>Retinal cells: formic acid (neurotoxin) | N/A | Blindness | Ethanol<br>Fomepizole |
| Methyldopa | Causes autoimmune reaction | N/A | Hemolytic anemia | None |
| Metoclopramide | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Mold spores | Cause type I, type III hypersensitivity reaction | N/A | Allergic rhinitis; asthmatic bronchoconstriction; hypersensitivity pneumonitis | Antihistamines<br>Glucocorticoids |
| Morphine | Opioid agonist | Opioid receptors | Respiratory depression, constipation, nausea, vomiting, sedation, euphoria, peripheral vasodilation, urinary retention, miosis | Naloxone |
| MPTP | Converted to $MPT^+$, a neurotoxin | N/A | Parkinsonian symptoms | None |
| Nitric acid | Causes type I hypersensitivity reaction | N/A | Asthmatic bronchoconstriction | None |
| Nitrites | Oxidize hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |

*(continued)*

## TABLE 47-1 Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Nitrogen mustards | Alkylate DNA | N/A | Skin blistering; carcinogenesis | None |
| Nitroglycerin | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Oral contraceptives | Modulate transcription | Steroid receptors | Deep vein thrombosis; hepatic adenoma; gallbladder disease | None |
| Parathion | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Pentadecacatechol | Causes type IV hypersensitivity reaction | N/A | Contact dermatitis | None |
| Pet dander | Crosslinks IgE; causes type I hypersensitivity reaction | N/A | Allergic rhinitis; asthma | Antihistamines<br>Glucocorticoids |
| Platinum chloride | Crosslinks IgE; causes type I hypersensitivity reaction | N/A | Asthmatic bronchoconstriction | None |
| Primaquine | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Procainamide | Causes autoimmune reaction by inducing autoantibodies against DNA | N/A | Lupus-like syndrome | None |
| Sarin | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |

*(continued)*

**TABLE 47-1** Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Silica | Inhaled, fine particles of crystalline silica are deposited in the lungs, eliciting an inflammatory reaction | N/A | Pulmonary fibrosis; lung cancer; scleroderma | None |
| Soman | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Sulfonamides | Oxidize hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Sulfur dioxide | Causes type I hypersensitivity reaction | N/A | Asthmatic bronchoconstriction | None |
| Sulfuric acid | Strong acid | N/A | Chemical burns | None |
| Tabun | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Talc | Inhaled particulates cause granuloma formation | N/A | Pulmonary fibrosis | None |
| Tetrachloroethylene (perchloroethylene) | Converted to reactive species via the action of cytochrome P450 | N/A | CNS depression (acutely); possible carcinogenesis | None |
| Tricyclic antidepressants | Quinidine-like effect on sodium channel; lengthen QRS interval | Sodium channels | Ventricular dysrhythmias, hypotension, heart block, bradyarrhythmias, asystole | Sodium bicarbonate |

*(continued)*

TABLE 47-1 Mechanisms of Toxicity, Receptors, Clinical Signs, and Antidotes of Selected Poisons *(Continued)*

| POISON | MECHANISM OF TOXICITY | RECEPTOR (IF APPLICABLE) | CLINICAL SIGNS (ALL REQUIRE SUFFICIENTLY HIGH EXPOSURES/DOSES, AND ARE NOT EXPECTED IN ALL SETTINGS) | ANTIDOTE (IF AVAILABLE) |
|---|---|---|---|---|
| Trinitrotoluene | Oxidizes hemoglobin to methemoglobin | Hemoglobin | Cyanosis, nausea, vertigo, vomiting, collapse, tachycardia, tachypnea, coma, convulsions, and death | Methylene blue |
| Urushiol | Causes type IV hypersensitivity reaction | N/A | Contact dermatitis | Glucocorticoids |
| VX | Inhibits acetylcholinesterase | Acetylcholinesterase (directly); acetylcholine receptors (indirectly, by increasing concentration of acetylcholine at the synapse and neuromuscular junction) | Nausea, vomiting, diarrhea, hypersalivation, muscle paralysis, bradycardia | Atropine<br>Pralidoxime |
| Warfarin | Prevents clotting factor synthesis by inhibiting vitamin K regeneration | Epoxide reductase | Bleeding; teratogenic, causing facial defects | Vitamin K<br>Frozen fresh plasma |

within approximately 15–30 minutes of administration. Locally, it irritates the gastrointestinal tract, while centrally it activates the chemoreceptor trigger zone (see Chapter 12, Pharmacology of Affective Disorders) in the area postrema of the brain. Alternatively, the dopamine agonist **apomorphine** can be given subcutaneously, which is preferable in an unresponsive patient. Apomorphine works directly on the chemoreceptor trigger zone by activating the dopamine receptors in the emesis center. **Chemical adsorption** of a toxin onto **activated charcoal** is another way to prevent the absorption of ingested toxins. Activated charcoal powder provides a large surface area onto which many small organic molecules can be adsorbed. The charcoal then passes through the gastrointestinal system and is eliminated in the feces, along with the adsorbed toxin. In general, activated charcoal is more effective for hydrophobic substances, and is generally ineffective for most inorganic salts. In some cases, **multiple-dose activated charcoal** can be given to interfere with enterohepatic circulation of drugs that have been systemically absorbed.

## Inhibition of Toxication

In some cases, it is possible to prevent toxication by inhibiting the enzymes involved in metabolizing the xenobiotic into its toxic product. In practice, few specific metabolic enzymes are targets of therapy because most of the enzymes are part of the cytochrome P450 system, which, as discussed in Chapter 4, are not substrate specific.

However, one target for such an approach is **alcohol dehydrogenase,** which converts alcohols into their corresponding aldehydes. In many cases, the parent alcohol is not particularly toxic and can be excreted renally, but the aldehyde metabolite or a more downstream metabolite is significantly more toxic. For example, formate is a toxic metabolite of methanol (wood alcohol), while glycolic acid is the toxic metabolite of ethylene glycol (a bifunctional alcohol and a component of antifreeze) that causes dose-dependent CNS depression, acute renal failure, and elevated anion gap metabolic acidosis. Because **ethanol** is also metabolized by alcohol dehydrogenase, it can function as a competitive inhibitor for both methanol and ethylene glycol. Historically, patients who had ingested methanol or ethylene glycol were kept inebriated with oral or IV ethanol solutions to minimize the formation of the more toxic metabolites. More recently, the competitive alcohol dehydrogenase inhibitor **fomepizole** has been employed. Unlike ethanol, fomepizole is not itself metabolized by alcohol dehydrogenase and does not cause symptoms of inebriation.

## Enhancement of Metabolism (Detoxication)

It is theoretically possible to accelerate metabolism of a toxic substance by inducing the appropriate cytochrome P450 isoenzyme. Unfortunately, because the process of induction takes time, this approach is not suitable for acute intoxications and is not generally used clinically.

In some cases, the metabolic actions of non-P450 enzymes dependent on cofactors or cosubstrates may be accelerated by the addition of these cofactors. The most dramatic example is cyanide poisoning, which is treated with a "kit" containing **amyl nitrite, sodium nitrite,** and **sodium thiosulfate** (Fig. 47-5). The nitrites act by oxidizing hemoglobin to methemoglobin to provide a substrate that can compete with cytochrome *c* oxidase for cyanide molecules (see below). The methemoglobin-bound cyanide is then oxidized to the relatively nontoxic thiocyanate by the enzyme **rhodanese** (also known as transsulfurase). The addition of thiosulfate provides a ready source of sulfur for the detoxication reaction and enhances cyanide metabolism.

Another example of providing material needed for a detoxification reaction is the use of N-acetylcysteine in the treatment of **acetaminophen** poisoning. As discussed previously, acetaminophen can be converted into the toxic metabolite N-acetylbenzo-*p*-quinoneimine (NAPQI) by the action of cytochrome P450 (see Fig. 47-6). NAPQI can be detoxified by conjugation with glutathione. However, if the acetaminophen dose is large enough, glutathione stores are depleted. Glutathione stores can be replenished by administering **N-acetylcysteine** (NAC), a metabolic precursor of glutathione.

## Enhancement of Elimination

Facilitating toxin elimination is a common method of treating an acutely poisoned patient. This can be accomplished either by enhancing renal clearance by preventing tubular reabsorption (**ion trapping**), or by artificially clearing the drug from plasma by means of **hemodialysis, hemofiltration,** or **hemoperfusion.**

Ion trapping involves alkalinization of the urine to enhance renal clearance of a weakly acidic toxin. In ion trapping, the neutral form of the toxin to be eliminated is filtered through the glomerulus and becomes deprotonated in the basic urine. The ionized form of the toxin is not reabsorbed and is excreted in the urine. Clinically, ion trapping is accomplished by administering bicarbonate to the patient and titrating to a urine pH of 7.5 to 8.5. The technique has been effective in improving the elimination of salicylates and phenobarbital. Although acidification of urine to enhance clearance of a basic toxin is theoretically possible, the practical dangers of iatrogenic metabolic acidosis preclude the use of this approach.

The techniques of hemodialysis, hemofiltration, and hemoperfusion depend on extracorporeal purification of the blood. For this reason, the techniques only work with substances that have a relatively small volume of distribution, so that removal of the toxic substance from the blood does not leave a large, inaccessible reservoir in the body tissues. Hemodialysis functions by passing the blood through a chamber where it is separated from dialysis fluid by a semipermeable membrane. The membrane allows small molecules to diffuse from the higher concentration found in the plasma to the lower concentrations found in the dialysis fluid. Hemodialysis is generally useful for small molecules that have a small volume of distribution, are relatively water soluble, and are not highly bound to plasma proteins (which cannot cross the dialysis membrane).

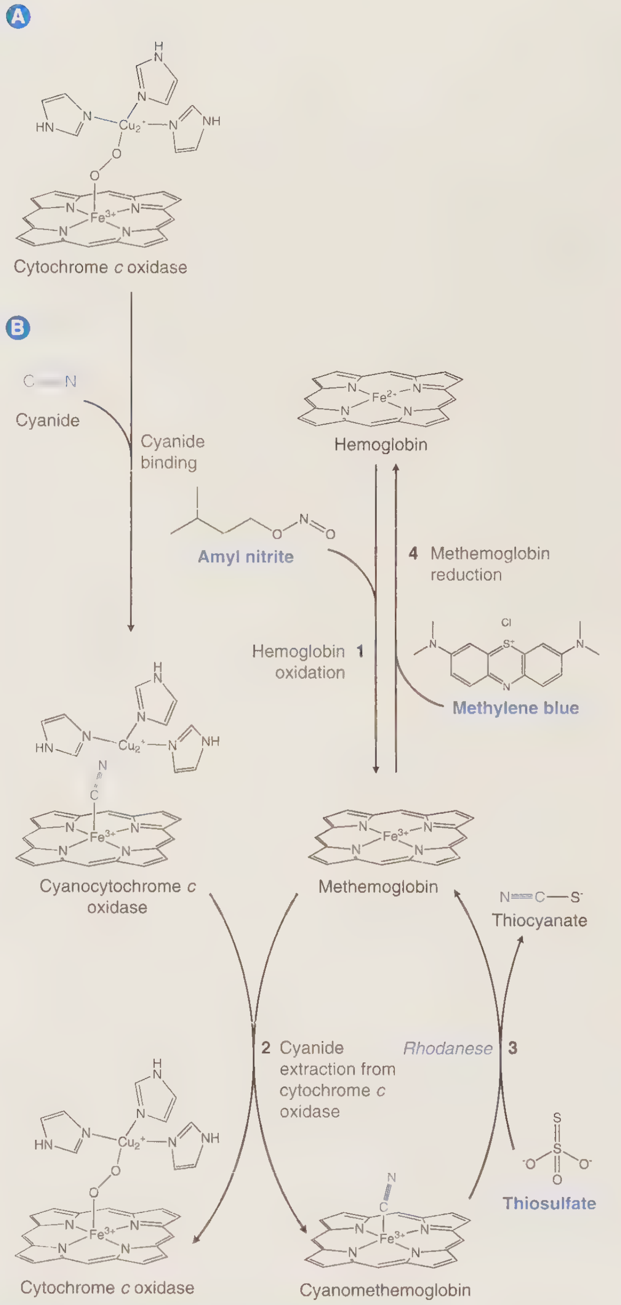


**Figure 47-5. Treatment of Cyanide Poisoning. A.** Structure of the copper/heme active site of cytochrome *c* oxidase, the enzyme responsible for the final step of the electron transport chain (the four-electron reduction of oxygen to water). Here, the enzyme is depicted after reducing oxygen to the bridging peroxide form. **B.** Cyanide displaces oxygen because it forms an extremely stable bond with the ferric heme group in cytochrome *c* oxidase. Treatment for cyanide poisoning involves (1) oxidation of the ferrous heme in hemoglobin to its ferric form (methemoglobin) by amyl nitrite or sodium nitrite. Methemoglobin competes strongly for cyanide (2), facilitating its removal from the active site of cytochrome *c* oxidase, and acting as a sink for circulating cyanide. Cyanide is converted to thiocyanate via the action of rhodanese, a mitochondrial enzyme (3). The addition of sodium thiosulfate provides the sulfur needed for the conversion of cyanide to thiocyanate. Once cyanide has been detoxified, methemoglobin can be returned to its ferrous form (4) by the addition of methylene blue.

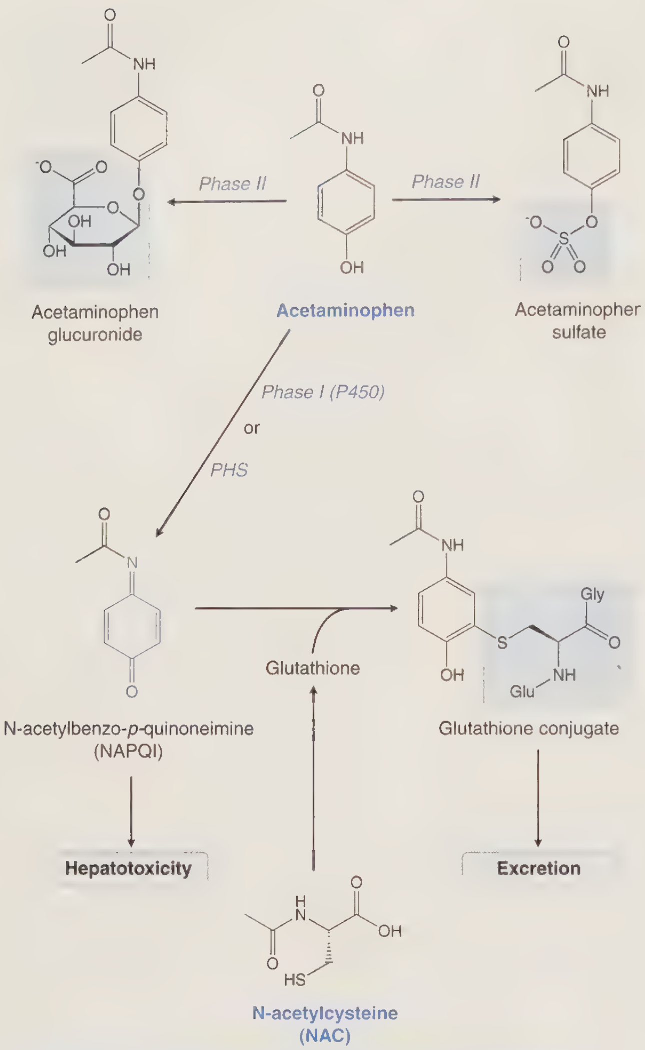


**Figure 47-6. Mechanism of Acetaminophen Poisoning and Treatment.** Acetaminophen is not, itself, toxic, but can be converted to toxic metabolites in the liver by the oxidative action of cytochrome P450 or prostaglandin H synthase (PHS). Most acetaminophen is conjugated to sulfate or glucuronate via phase II reactions. However, a small amount is oxidized to N-acetylbenzo-*p*-quinoneimine (NAPQI), which can bind to hepatic proteins and cause centrolobular necrosis (hepatotoxicity). NAPQI can be conjugated to glutathione to form the nontoxic glutathione conjugate. In cases of acetaminophen overdose, glutathione is depleted, and NAPQI is free to cause heptotoxicity. N-acetylcysteine (NAC) can be administered as an antidote. NAC, which is a metabolic precursor for glutathione, repletes hepatocellular glutathione levels and, thereby, prevents NAPQI-induced hepatotoxicity.

In hemofiltration, the blood is filtered through a variable-sized porous membrane, which allows plasma with a varying amount of protein to be removed in the ultrafiltrate. Because the plasma is exposed to a filter rather than a semipermeable membrane, larger molecules can be cleared with hemofiltration than hemodialysis. In addition, because proteins can be allowed to pass through the filter, toxins with higher protein-binding characteristics can successfully be cleared. On the other hand, hemofiltration requires the replenishment of plasma components that have been eliminated in the ultrafiltrate.

In hemoperfusion, blood is passed through a cartridge where it comes in contact with an **ion-exchange resin** or activated charcoal that can adsorb the toxin. As discussed above, charcoal generally adsorbs hydrophobic molecules. Ion-exchange resins bind to inorganic salts and exchange them for electrolytes such as sodium or chloride. Like hemodialysis and hemofiltration, hemoperfusion requires that the toxin have a low volume of distribution. However, unlike hemodialysis, hemoperfusion does not require that the toxin have a low protein binding because the equilibrium strongly favors adsorption to the adsorbent. Because of the direct contact between blood and the adsorbent material, hemoperfusion poses a greater risk of clotting. In addition, the ion-exchange resin is not selective in its ion binding and can deplete plasma calcium and magnesium.

In some cases, an ion-exchange resin can be given orally, to bind ions that are normally present in the gastrointestinal system. For example, elevated potassium levels can be reduced through the administration of **sodium polystyrene sulfonate,** which binds potassium and is then eliminated in the feces.

In extreme circumstances, it may be possible to conduct a **plasma exchange** or an **exchange transfusion,** whereby the patient's plasma or blood is removed and replaced with transfused plasma or whole blood from a donor. This technique is generally reserved for newborns.

## INACTIVATION OF POISONS

One approach to reducing the activity of toxins that have entered the circulation and cannot be eliminated easily is **inactivation.** A toxin is inactivated by binding it to a substance so that it is unable to interact with and damage the target tissues. The complexes of toxin and inactivator are then cleared by the body. In some cases, the inactivator even facilitates clearance of the toxin. An inactivating substance must have a high affinity for the toxin so that equilibrium strongly favors the inactivated complex, and the complex formed must have a low toxicity. Inactivating substances can be small molecules or macromolecules, usually antibodies or antibody fragments.

### Heavy Metal Chelators

The inactivation and removal of toxic heavy metals such as lead, mercury, or cadmium, and overdoses of metals such as iron or copper, can be accomplished by binding them to a small molecule containing a nucleophilic electron donor such as an amine, hydroxide, carboxylate, or mercaptan to form a **metal–ligand complex.** A **chelator,** which in Greek means "claw," is a multidentate structure with multiple binding sites (Fig. 47-7). Binding the metal at multiple sites shifts the equilibrium constant in favor of metal binding. A high affinity binding for the metal is critical because the chelator must compete with tissue macromolecules for binding. In addition, the chelator should be nontoxic, water-soluble, and the complex should be easy to clear. Finally, an ideal chelator should have a low binding affinity for endogenous ions such as calcium. Unfortunately, the high concentration of calcium in certain tissues means that equilibrium often favors calcium binding. To prevent the depletion of tissue calcium, many chelators are administered as their calcium complexes. The target metal is then exchanged for calcium, and the body's calcium stores are not depleted.

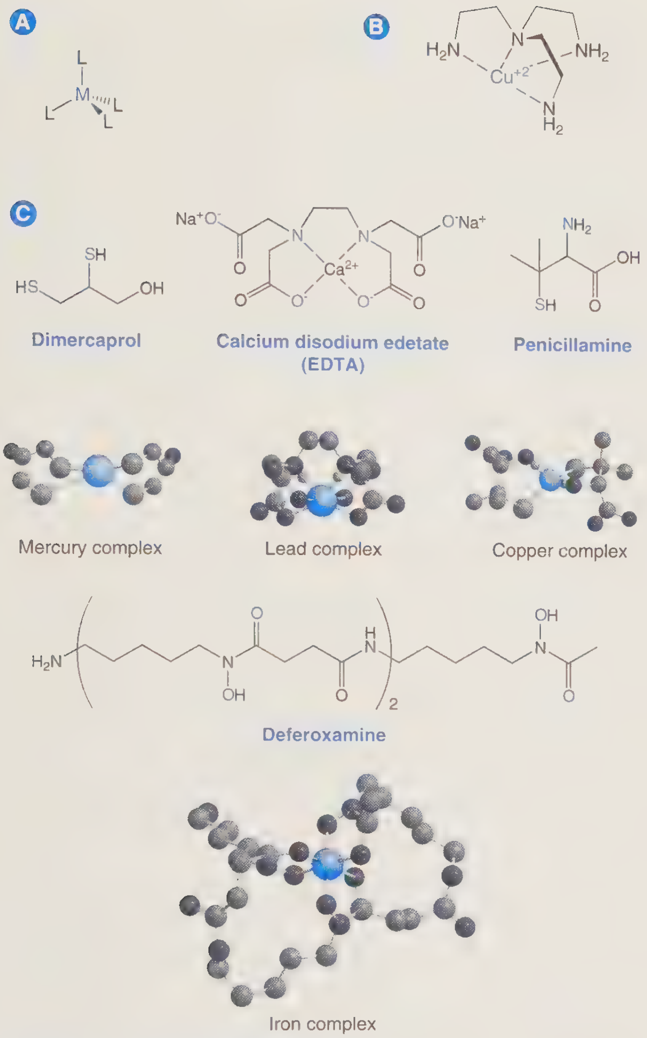


**Figure 47-7. Heavy Metal Chelators.** **A.** A ligand (L) is a compound containing a Lewis base (such as amine, thiol, hydroxyl, or carboxylate groups) that can form a complex with a metal (M). **B.** A chelator is a multidentate ligand, that is, a ligand that can bind to a metal through multiple atoms, as in this example of a tetraamino ligand bound to copper ($Cu^{2+}$) via its four amine groups. **C.** The structures of dimercaprol, calcium EDTA, penicillamine, and deferoxamine are shown; the groups that form bonds with the metal are identified in blue. Three-dimensional structures of the mercury complex of dimercaprol, the lead complex of EDTA, the copper complex of penicillamine, and the iron complex of deferoxamine are also shown. Here, the heavy metal is highlighted in blue. For simplicity, hydrogen atoms are not shown.

The most important heavy metal chelators are **edetate disodium** (the calcium, disodium complex of EDTA), which can be used to bind lead, **dimercaprol** (also known as **British anti-Lewisite** or BAL), which binds gold, arsenic, lead, and mercury to its two thiol groups, and **succimer** (2,3-dimercaptosuccinic acid), which has supplanted dimercaprol for the removal of lead, cadmium, mercury, and arsenic. **Deferoxamine** is used for the removal of toxic levels of iron, as would occur in the accidental overdose of iron-containing supplements or in patients who undergo chronic transfusion, such as those with sickle cell disease or thalassemia. Removal of copper, usually in patients with Wilson's disease, is accomplished with **penicillamine** or, for patients who do not tolerate penicillamine, **trientine.**

### Antivenins and Antibody Binding

Antibodies are also used as inactivators because they can have a high affinity and high specificity for their substrates. **Antivenins** are antibodies against a venom. They are produced by inoculating an animal, usually a horse, with small amounts of the venom to incite a humoral antibody response. The horse's purified serum is then injected into a patient who has been exposed to the venom, so that the antibodies contained in the serum may bind to and inactivate the venom.

The binding of digoxin with **digoxin immune Fab** in cases where plasma levels of the drug are toxic is another use of antibodies as inactivators. As the name implies, the antibody includes only the fab fragment of an immunoglobulin specific for digoxin derived from immunized sheep.

One danger with using antibodies as inactivators is the risk of developing serum sickness, a type III hypersensitivity reaction, described above. Both antivenins and digoxin immune fab can cause serum sickness.

## PHARMACOLOGIC TREATMENT

For toxins that act via a specific metabolic pathway, treatment can be rendered by administering a drug with opposite pharmacologic actions, or by bypassing the metabolic pathway that has been inhibited. There are four broad categories of pharmacologically mediated treatments used to counteract poisonings. A receptor antagonist can block the effect of a toxin that acts as an agonist at the receptor or that potentiates the action of the receptor's endogenous ligand. In cases where the action of the toxin is to antagonize a receptor, administering a receptor agonist or a drug that enhances the physiologic action of the endogenous ligand can restore balance at the receptor. Alternatively, a drug can restore the physiologic function of an enzyme or receptor that has been inhibited or distorted by a toxin. Finally, it is sometimes possible to bypass the metabolic pathway completely by taking advantage of pharmacologic targets downstream.

### Pharmacologic Antagonism

Conceptually, the simplest treatment for a poisoning is the administration of an antagonist that can block the action of a toxin that, directly or indirectly, results in superphysiologic activation of a receptor. One such example is the detoxification of opiates (see Chapter 16). Administration of **naloxone,** a pharmacologic antagonist of the opioid receptor, can inhibit the action of the opioid. This drug can also be used to reverse opiate overdoses, both those caused by the illicit use of a drug such as heroin, and iatrogenic overdoses, such as might occur in the overaggressive use of a patient-controlled analgesic. Naloxone has a fast onset of action and a high potency, so that if no improvement is seen within 10 minutes with doses of up to 10 mg, a different diagnosis or multiple toxic entities should be considered. Naloxone has a relatively short half-life, so it must be given every 1–4 hours to provide antagonism while the opiate is cleared.

**Flumazenil** is a pharmacologic antagonist of benzodiazepine receptors and is used to treat overdoses of these drugs. Like naloxone, flumazenil has a fast onset and high potency; its effects should be seen within 5 minutes at a dose of not more 3 mg. Like naloxone, flumazenil has a short half-life (approximately 1 hour) and must be given frequently to provide antagonism while the benzodiazepine is cleared.

Pharmacologic antagonism can also be used when the toxic agent is not a direct agonist but indirectly increases the concentration of the natural ligand for a receptor. Acetylcholinesterase inhibitors, discussed above, produce a superphysiologic concentration of acetylcholine in the synaptic cleft and a characteristic toxidrome of cholinergic excess: bradycardia, miosis, hypersalivation, sweating, diarrhea, vomiting, bronchoconstriction, weakness, respiratory paralysis, and convulsions.

Although, in some cases, it is possible to restore acetylcholinesterase (see below), the treatment of acetylcholinesterase inhibition generally depends on administering an anticholinergic agent, such as **atropine.** By antagonizing the muscarinic acetylcholine receptor, atropine restores cholinergic balance and prevents bronchoconstriction, the most common cause of death in patients with exposure to acetylcholinesterase inhibitors. Unfortunately, atropine does not antagonize nicotinic receptors and cannot reverse muscular paralysis. Thus, if exposure is such that the diaphragm is paralyzed, an agent that can displace the acetylcholinesterase inhibitor (see below) or supportive measures, such as mechanical ventilation, must also be instituted.

## Pharmacologic Enhancement of Physiologic Function

When the toxin is a competitive receptor antagonist, treatment can be effected by administering an agonist or a compound that enhances the physiological function. For example, ingestion of berries or seeds (such as from Jimson weed) that contain **belladonna alkaloids** causes an atropine-like anticholinergic effect that can result in delirium, coma, and respiratory collapse. Treatment involves the administration of **physostigmine,** which blocks acetylcholinesterase to increase cholinergic tone. It should be noted that the treatment for organophosphate inhibition of acetylcholinesterase is to give atropine, an anticholinergic agent, whereas the treatment for poisoning with an anticholinergic toxin is to administer an acetylcholinesterase inhibitor.

## Active Site Restoration

It is possible to treat many receptor antagonists, enzyme inhibitors, and disabled active sites by using a pharmacologic agent that can displace the toxic agent and restore enzyme or receptor activity. This strategy is best illustrated by the treatment for toxic agents that bind or oxidize heme groups. The three prototypic agents that act on heme groups are carbon monoxide (which acts on hemoglobin), cyanide (which acts on cytochrome *c* oxidase), and xenobiotics such as nitrate, a common water contaminant in rural areas (which is reduced in the body to nitrite that then oxidizes hemoglobin to methemoglobin).

Treatment for carbon monoxide and cyanide poisonings involves displacing the small molecule from its heme target. For carbon monoxide poisoning, high concentrations of oxygen are administered. The oxygen competes with carbon monoxide for hemoglobin, and the free carbon monoxide is exhaled. Higher concentrations of oxygen result in greater displacement and, therefore, more rapid elimination of carbon monoxide. In cases of severe poisoning, a patient may be placed in a **hyperbaric oxygen chamber** that delivers oxygen at partial pressures above atmospheric pressure. Hyperbaric oxygen also increases the blood's oxygen-carrying capacity by increasing the amount of oxygen dissolved in plasma, which complements the delivery of oxygen by hemoglobin.

Cyanide cannot be displaced by oxygen. However, cyanide does have a greater affinity for methemoglobin than for cytochrome *c* oxidase (see Fig. 47-5). Thus, by oxidizing hemoglobin to its ferric state (methemoglobin) with amyl nitrite or sodium nitrite, a competitor for cyanide is generated. Cyanide is then converted to thiocyanate with the aid of sodium thiosulfate. Once the danger of cyanide toxicity has passed, the ferric iron in methemoglobin can be reduced to its ferrous state.

To restore ferric methemoglobin to its ferrous state, an active redox agent, **methylene blue,** must be administered. Typically, the concentration of methemoglobin is kept low via two reducing pathways, the NADH diaphorase pathway, which normally accounts for > 95% of the methemoglobin reducing activity, and the NADPH diaphorase pathway, which accounts for the remainder. Methylene blue is reduced to leukomethylene blue by NADPH diaphorase and can then reduce methemoglobin to hemoglobin, restoring its oxygen carrying capacity.

The treatment for organophosphate poisonings also involves restoration of the active site of the enzyme. While the administration of anticholinergic agents such as atropine can block the effect of excess acetylcholine at muscarinic receptors, it cannot restore enzymatic function of acetylcholinesterase. However, **pralidoxime** can enhance the hydrolysis of the serine-phosphate bond between the organophosphate and acetylcholinesterase. Pralidoxime contains a quaternary ammonium group that places an oxime nucleophile in close proximity with the electrophilic phosphate group on the organophosphate (see Fig. 47-2). The organophosphate then binds to pralidoxime, releasing acetylcholinesterase. The resulting phosphorylated oxime is unstable in water and degrades. Unfortunately, once the phosphorylated acetylcholinesterase has undergone "aging" through the hydrolysis of an alkyl side-group, it is resistant to pralidoxime, so the drug should be given as soon as possible after exposure.

## Metabolic Pathway Alternatives

In some cases, it may be possible to bypass a toxin-inhibited enzymatic reaction entirely, either by supplying the enzymatic product or enhancing an alternative metabolic pathway. A common example of this therapeutic strategy is the administration of **vitamin K** in cases of certain **anticoagulant** poisonings. **Coumadin,** discussed in Chapter 21, Pharmacology of Hemostasis and Thrombosis, and certain anticoagulants used as rodenticides including **brodifacoum, diphacinone,** and derivatives, inhibit the regeneration of vitamin K from its epoxide. Vitamin K is needed for the carboxylation of glutamate to form $\gamma$-carboxyglutamate, converting precursor coagulation factors into their active forms. Hence, the depletion of reduced vitamin K stores results in the depletion of coagulation factors, prevents thrombosis, and facilitates bleeding. The treatment for overdose or poisoning with these anticoagulants, then, is the administration of supplementary vitamin K to enable the liver to generate the needed coagulation factors. However, because it takes time for the liver to synthesize coagulation factors, vitamin K therapy generally requires several hours to improve coagulation function. Thus, it may be necessary to bypass the entire metabolic pathway and administer to the patient **fresh frozen plasma** (FFP), which contains the necessary preformed coagulation factors. In cases of rodenticide poisoning, in which the anticoagulant has a long clearance time, it may be necessary to give multiple doses of vitamin K.

## *Conclusion and Future Directions*

Much of the treatment for toxic exposures focuses on the acutely poisoned patient. However, most of the morbidity associated with toxic exposures is caused by chronic exposures and is frequently manifested many years after the initial exposure, as was seen in the unfortunate case of Ms. Price. In fact, there is generally no specific treatment for injury caused by chronic toxic exposures, and much of the available treatment is symptomatic, supportive, and otherwise no different from the treatment administered for the same malady caused by other factors.

Thus, the study of toxicology includes not only **mechanistic toxicology,** on which this chapter has focused, but also **descriptive toxicology** and **regulatory toxicology.** Descriptive toxicology is concerned with the determination of what compounds are toxic and what their toxic effects are; regulatory toxicology helps develop public policy that reasonably minimizes exposure to toxic compounds. In the United States, several regulatory agencies are charged with forming such policy. The **Food and Drug Administration (FDA)** seeks to ensure the safety of the food supply, approve new therapeutic agents, and withdraw approvals of unsafe agents or medical devices (see Chapter 48, Drug Discovery and Development, and Chapter 51, New Drug Evaluation and Drug Regulation). The **Environmental Protection Agency (EPA)** establishes and enforces policy with regard to environmental pollution and its public health consequences. The **Occupational Safety and Health Administration (OSHA)** regulates workplace exposures, while the **Consumer Products Safety Commission (CPSC)** works to ensure the safety of consumer goods. The application of mechanistic understanding of toxicity is expected to improve regulatory toxicology and thus enhance public health.

## *Suggested Readings*

Klaassen, CD, ed. Casarett & Doull's Toxicology: The Basic Science of Poisons. 6th ed. New York: McGraw-Hill, 2001. (*A comprehensive textbook of toxicology, this resource provides a solid foundation for the understanding of toxicology. It includes sections on general principles, toxicokinetics, nonspecific toxicity, organ-specific toxicity, toxic agents, environmental toxicology, and applications of toxicology, including a chapter on clinical toxicology.*)

Nelson E. The miseries of passive smoking. Hum Exp Toxicol 2001;20:61–83. (*Discusses the significant health risks associated with passive cigarette smoke.*)

Robbins SL, Kumar V, Cotran RS, et al. Robbins Pathologic Basis of Disease. 6th ed. Philadelphia: WB Saunders, 1999. (*This textbook of pathology covers the pathophysiology of toxic and environmental exposures, as well as the pathology and pathophysiology of organ system toxicity.*)

Smilkstein MJ, Knapp GL, Kulig KW, et al. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med 1988;319:1557–1562. (*This study established the clinical benefit of treating acetaminophen toxicity by providing N-acetylcysteine, a source of cysteine for the production of glutathione.*)

Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol 2002;78:31–41. (*Overview of common sources of food poisoning.*)

Toxnet (http://toxnet.nlm.nih.gov/) (*This government resource, sponsored by the National Library of Medicine, contains a vast database of both toxic substances and articles in the field of toxicology.*)

Vogel RA. Alcohol, heart disease, and mortality: a review. Rev Cardiovasc Med 2002;3:7–13. (*Reviews the major mechanisms of alcohol toxicity on the heart.*)

Weaver LK, Hopkins RO, Chan KJ, et al. Hyperbaric oxygen for acute carbon monoxide poisoning. N Engl J Med 2002;347:1057–1067. (*Although hyperbaric oxygen had been postulated to help treat carbon monoxide poisoning and has been used since 1960, this study established its clinical efficacy in reducing cognitive deficits at 6 weeks and 12 months.*)

# VIII

# Contemporary Approaches to Drug Discovery, Development, and Delivery

# 48

# Drug Discovery and Development

Joshua M. Galanter and Christopher T. Walsh

**Introduction**
**Case**
**Drug Discovery**
  Compound-Centered Drug Design
    *Natural and Synthetic Compounds*
    *Agonist Analogues*
  Target-Centered Drug Design
    *High-Throughput Screening*
    *Combinatorial Chemistry*
    *Structure-Based Drug Design*
  Peptides, Peptidomimetics, and Macromolecules
    *Lead Optimization*
**Drug Development**
  Biological Characterization
  Chemical Characterization
  Chemical Synthesis and Manufacturing
    *Chemical Synthesis*
    *Scale-up*
    *Macromolecule Development*
    *Manufacturing*
  Formulation
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Over the last decade, some 370 new drugs have been approved by the United States Food and Drug Administration (FDA). Many such drugs have enabled treatments for diseases that were previously untreatable. Others have yielded expanded treatment options because they are more efficacious, less toxic, or both. In the fight against infectious diseases, pharmaceutical companies, university laboratories, and others continue to develop new agents to fight diseases that have become resistant to existing treatments. With new technologies and information from the human genome project, it is hoped that significant new classes of drugs will continue to be discovered in the coming decades.

The development of a new drug is not easy. It is quite costly, and few drugs that begin development are approved for marketing. Of 10,000 compounds considered promising from results in initial screening assays, fewer than 10 make it to clinical trials, and only two are eventually approved. The development of new drugs is a risky venture, but, if successful, can be quite profitable for those willing to take such risks. The most successful drugs, such as **atorvastatin,** have annual sales of well over $3 billion.

In this chapter, the major methods used to discover new drugs are described. Then, the studies that must be conducted before a drug may be tested in the clinic are discussed. This includes the drug's chemical synthesis, manufacturing process, and formulation, as well as studies on the molecular mechanism of action, chemical properties, and biological and biochemical profile, such as the drug's efficacy in laboratory animals, toxicology data, and pharmacokinetics. Clinical studies are described in Chapter 51, New Drug Evaluation and Drug Regulation.

## Case

In 1987, researchers at Abbott Laboratories decided to target HIV protease in their search for a novel antiviral therapeutic. Abbott chose the protease because it is essential to HIV's replication and because it has an unusual substrate specificity (see Chapter 34, Pharmacology of Viral Replication). The natural substrate for the enzyme contains a phenylalanine-proline bond, a rare cleavage site for mammalian proteases, so researchers reasoned that a drug that inhibits HIV protease would have relatively few side effects.

In 1989, crystallographers at Merck announced that they had elucidated the crystal structure of HIV protease. Based on the newly solved structure, researchers now knew that the viral protease was a symmetrical dimer of two identical subunits (Fig. 48-1). Using a molecular model, researchers at Abbott designed an analogue of the enzyme's natural substrate by replacing the proline in the natural substrate with a phenylalanine and designing a symmetrical molecule containing identical side units on each end of

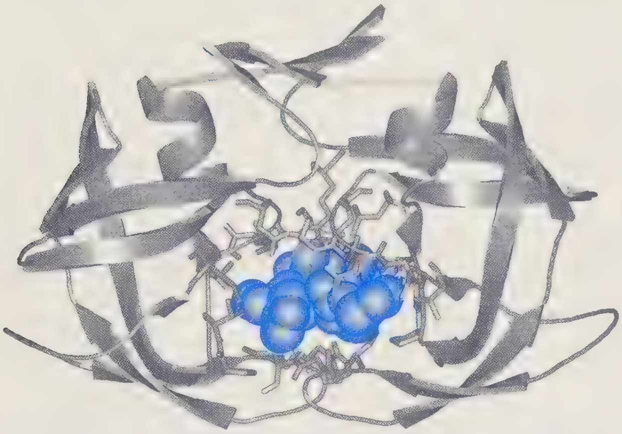


**Figure 48-1. Crystal Structure of HIV-1 Protease Bound by Ritonavir.** The structure of HIV protease is shown as a ribbon, with ritonavir (blue space-filling model) occupying the active site. The rotational axis of symmetry of the enzyme is evident and was the basis for the design of the drug. Using the crystal structure of HIV protease, researchers were able to fine-tune the structure of the inhibitor to achieve a $K_i$ of less than 5 nM (also, see Fig. 34-8).

the structure. They also replaced the peptide bond in the center of the molecule with a functional group that mimicked the transition state of the enzymatic reaction, but was resistant to cleavage by the protease. Although this first molecule was a weak inhibitor of viral protease, the researchers used knowledge about the enzyme's structure to add additional functional groups to the molecule that would likely increase its potency. The result was a drug that bound the enzyme with 10,000-fold higher affinity than the first structure, but, unfortunately, displayed poor pharmacokinetics.

Chemists continued to alter functional groups on the candidate drug until **ritonavir,** a highly potent molecule with acceptable pharmacokinetic properties, was created. Incidentally, studies of ritonavir in tissue culture showed that it inhibited a cytochrome P450 subtype involved in the metabolism of other candidate protease inhibitors.

Ritonavir was approved for marketing by the FDA in 1996. In 2000, working from pharmacokinetic and clinical studies that showed that ritonavir increased the bioavailability (see Chapter 3, Pharmacokinetics) of another protease inhibitor, **liponavir,** the FDA approved the marketing of a combination drug that contains both ritonavir and liponavir.

## QUESTIONS

- **1.** What tools can researchers use to "discover" new drugs such as ritonavir?
- **2.** How can structural information about a molecular target help in the drug discovery process?
- **3.** How do researchers evaluate drug candidates?
- **4.** How do drug development investigations help elucidate likely therapeutic features, such as the pharmacokinetics and toxicology of a drug?

## DRUG DISCOVERY

The term **drug discovery** refers to the process by which pharmaceutical, academic, or government laboratories identify or screen compounds to find potentially active pharmacologic agents. Screening consists of testing many compounds in an assay relevant to the disease in question: a compound that passes a screen is called a **hit.** If the compound or its structural derivatives continue to show promise after further biological and chemical characterization, it becomes a **lead.** Ideally, drug discovery should be cost effective and produce hits that have a high likelihood of conversion to leads and, eventually, successful drugs (Fig. 48-2).

Two basic approaches are used to identify hits. In a **compound-centered** approach, a compound is identified and its biological profile is explored. If the compound displays biological activity, it is refined and developed further. In a **target-centered** approach, the putative drug target is identified first. This may be a receptor thought to be involved in a disease process, a critical enzyme, or another biologically important molecule in the disease pathway. Once the target is identified, researchers search for compounds that interact with it. The search may be systematic, using information about the structure of the target as a starting point, or it may take a "**shotgun approach,**" whereby all the compounds in a large library of substances, synthesized via **combinatorial chemistry,** are tested in a high-speed automated assay. Modern drug discovery typically involves elements of both approaches. A hit initially found through a compound-centered approach is often modified with the aid of knowledge about its target; and, a high throughput screen designed with specific knowledge of a receptor can be used to test the biological activity of multiple potential drug molecules.

## COMPOUND-CENTERED DRUG DESIGN

### Natural and Synthetic Compounds

Traditionally, drug discoveries have resulted from a compound-centered approach. Many of the earliest drugs discovered were **natural products** isolated from plants, molds, or other organisms. Often, the discoveries were made serendipitously. For example, **penicillin** (see Chapter 35, Pharmacology of the Bacterial Cell Wall), was discovered when Alexander Fleming observed that spores of the contaminant mold *Penicillium notatum* inhibited bacterial growth. Other natural products that have been transformed into successful drugs are **paclitaxel,** a chemotherapeutic derived from the Pacific Yew tree, **morphine,** an opioid analgesic obtained from the opium poppy, **streptokinase,** a thrombolytic agent obtained from streptococcal bacteria, and **cyclosporine,** an immunosuppressive agent obtained from a fungus. Table 48-1 lists a number of drugs obtained from natural products.

There are several advantages to examining natural products as a source for active agents. First, natural products have a reasonable likelihood of biological activity. Second, it may be easier to isolate a compound from its natural source than to synthesize a compound de novo, especially if the structure of the compound is complex or requires difficult synthetic

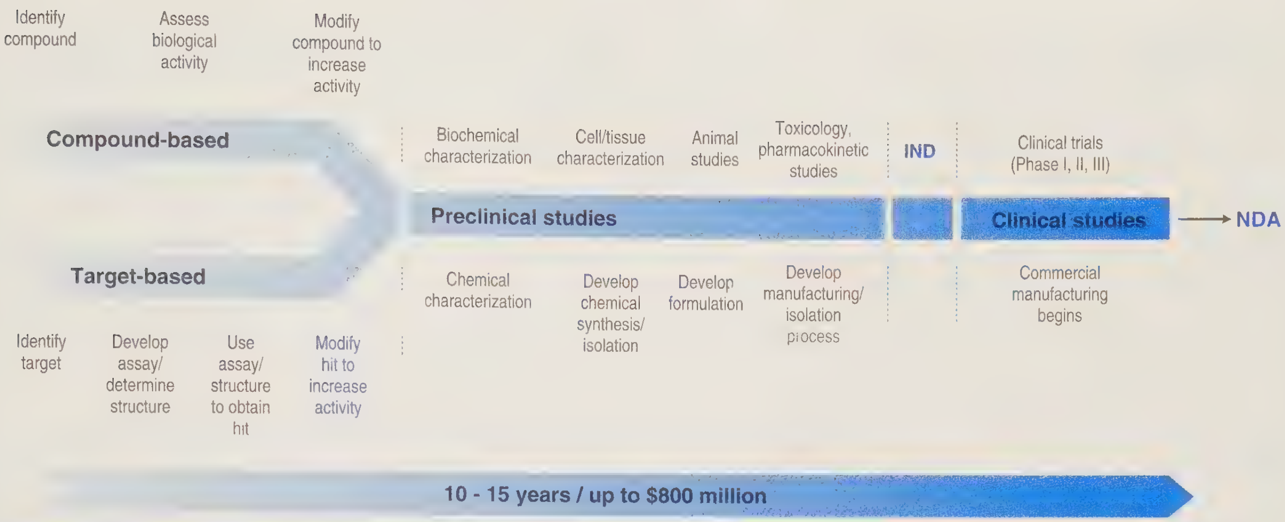

Identify compound
Assess biological activity
Modify compound to increase activity
Compound-based
Target-based
Identify target
Develop assay/ determine structure
Use assay/ structure to obtain hit
Modify hit to increase activity
Biochemical characterization
Cell/tissue characterization
Animal studies
Toxicology, pharmacokinetic studies
IND
Clinical trials (Phase I, II, III)
Preclinical studies
Clinical studies
NDA
Chemical characterization
Develop chemical synthesis/ isolation
Develop formulation
Develop manufacturing/ isolation process
Commercial manufacturing begins
10 - 15 years / up to $800 million

**Figure 48-2. Timeline for the Steps of Drug Discovery and Development.** The drug discovery process can proceed through either a compound-based or target-based approach; most modern approaches use elements of both. Concurrent with drug discovery, drug development involves chemical and biological characterization as well as synthetic optimization and manufacturing. These processes overlap considerably, and the results of iterative processes are used to improve the lead; this process is called lead optimization. Clinical trials and regulatory approval (Chapter 51) are also required before a drug may be marketed. The entire process from compound identification to drug approval can take 10–15 years and cost up to $800 million. IND, investigational new drug application. NDA, new drug application.

manipulations. Third, it may be feasible to use the natural compound as a starting point for synthetic fine-tuning, to form a semisynthetic product. Fourth, a natural product may be significantly more complex than a likely synthetic analogue and is generally isomerically pure. Paclitaxel, for example—the yew tree component—has a complex structure that contains four fused rings, one of which contains eight carbons. A chemical synthesis for the compound took over 50 steps to complete, and had a total yield of less than 1%. Of course, natural products also have disadvantages: it often takes significant effort to isolate a natural product, without a guarantee of success. Although there is a relatively high likelihood of biological activity compared to many synthetic compounds, it is often difficult to know what system or systems should be used for testing function. Even if it is found to be pharmacologically active, a natural product can be expensive to isolate and modify.

**Synthetic compounds** are also be used to search for new drugs. Researchers can construct a library consisting of thousands of compounds with differing structural characteristics, tailored for a particular type of investigation: a library may, for example, consist of numerous compounds with a phenylanine-proline bond. However, it may be impractical to synthesize large, complex molecules, and isomeric purity may be elusive.

## Agonist Analogues

An alternative compound-centered approach uses the natural agonist of a receptor or one of its precursors as a basis for drug development. For example, because lack of **dopamine** is associated with Parkinson's disease (see Chapter 11, Pharmacology of Dopaminergic Neurotransmission), one of the first treatments involved administering **L-DOPA** (the drug **levodopa**), a metabolic precursor of dopamine. **Insulin** was developed in much the same way; once it was discovered that the signs and symptoms of diabetes were caused by low insulin levels, insulin was administered exogenously as treatment.

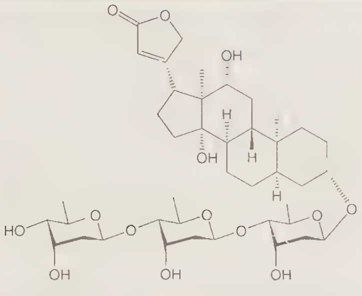
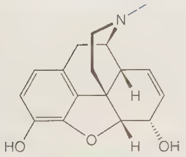
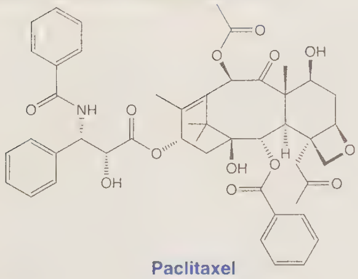
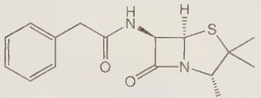
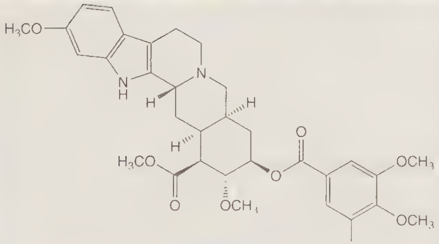
The natural agonist for a receptor can also serve as a skeleton on which chemical modifications can be made. These changes can alter the compound's binding affinity, physiologic effect (such as converting an agonist into an antagonist; see Chapter 1, Drug–Receptor Interactions), distribution, or pharmacokinetics. This approach was employed in the development of **cimetidine** (see Chapter 41, Pharmacology of Histamine), an $H_2$ receptor antagonist. Starting with histamine, researchers made successive modifications of the skeleton to synthesize an antagonist with high binding affinity for the receptor and decreased toxicity.

Modifying a small-molecule agonist has a relatively high likelihood of success. Because the natural agonist is biologically active, chemical derivatives thereof may well be effective if given exogenously. Of course, problems may also arise. Dopamine formed from exogenous L-DOPA can bind to receptors in nontargeted areas of the brain and cause hallucinations similar to those seen in psychosis. Moreover, many disease processes are not mediated by the interaction of a small-molecule agonist and its receptor. Many targets for pharmaceuticals are ion channels or proteins that interact with other proteins, and hence are not amenable to this agonist analogue approach.

## TARGET-CENTERED DRUG DESIGN

In a target-centered approach to drug discovery, researchers use a biochemical or molecular target to search for new hits. This approach has several advantages. First, if the target has been associated with a disease process, a hit that successfully interacts with the target has a relatively high likelihood of useful pharmacologic activity. Second, because the target is

TABLE 48-1 Examples of Natural Products Used as Drugs, Their Sources and Uses

| NAME (GENERIC)/STRUCTURE | INDICATION (SEE CHAPTER) | SOURCE |
|---|---|---|
| **Cyclosporine** | Immunosuppressant (Chapter 43) | *Beauveria nivea* (fungus) |
|  **Digoxin** | Antiarrhythmic, cardiac inotrope (Chapters 17, 18, 23) | *Digitalis lanata* (white foxglove), *Digitalis purpurea* (purple foxglove), numerous other plants |
|  **Morphine** | Analgesic (Chapter 15) | *Papaver somniferum* (poppy plant) |
|  **Paclitaxel** | Cancer chemotherapeutic (Chapter 32) | *Taxus brevifolia* (Pacific yew tree) |
|  **Penicillin G** | Antibacterial (Chapter 35) | *Penicillium chrysogenum* (mold) |
|  **Reserpine** | Antihypertensive (Chapter 23) | *Rauwolfia serpentina* (plant) |
| **Streptokinase** | Thrombolytic (Chapter 21) | Beta-hemolytic streptococci (bacteria) |

The structures of streptokinase and cyclosporine are extremely complex, and therefore are not included in this table.

known, it may be easier to carry out assays that isolate the effect of an agent on the target. This is especially true for disease processes too complex to observe in cell or tissue preparations. For example, a drug's effect on atherosclerosis may be difficult to measure. However, it is relatively easy to measure whether a potential drug inhibits, for example, HMG-CoA reductase, an enzyme crucial in the cholesterol biosynthesis pathway (see Chapter 22, Pharmacology of Cholesterol and Lipoprotein Metabolism). As knowledge of the pathophysiology of disease processes has increased, target-centered approaches to drug discovery have become more successful, and more new drugs have been discovered using target-centered methods. HIV protease inhibitors, such as ritonavir, are notable examples of a drug class discovered with a target-centered approach.

## High-Throughput Screening

The simplest target-centered approach involves rapidly screening many molecules using an assay based on the drug target. **High-throughput screening,** using robotic automation, allows the rapid screening of thousands of compounds quickly.

Two aspects are critical in this approach. First, a large library of compounds must be available for screening. Second, a robust assay that leads to rapid identification of real hits must be developed. The assay may be as simple as a study to determine the binding affinity of drug candidates for a receptor (see Chapter 2, Pharmacodynamics), or may be more sophisticated, involving more complicated biochemical or cell-based manipulations. The library is then "run through" the assay, and any hits are examined more closely. An assay run in a **96 or 384 well plate** allows researchers to screen at least 96 or 384 compounds simultaneously. Any compound that produces a hit may display useful pharmacologic activity, because its biological target is used to screen for hits. In addition, once a library of compounds has been established, the same library can be run through many different assays. Of course, the quality of the results is dependent on the quality of the assay and the compounds in the library, so that a limited library or a poorly designed assay may result in false hits or may miss viable candidates. Because high-throughput screening places a premium on fast assays, false positives and false negatives are likely. Even when a hit is found, it will most likely need to be refined to increase its binding affinity or to change its pharmacologic properties (specificity, solubility, kinetics, etc.) this process is called "**hit-to-lead development.**"

## Combinatorial Chemistry

One refinement to the process of high-throughput screening has been the relatively recent introduction of **combinatorial chemistry.** Combinatorial chemistry uses a relatively small number of precursor molecules to generate a large number of compounds, analogous to how nature constructs a wide variety of proteins from a relatively small number (~20) of amino acids. Researchers are not limited to natural substances, but generally use a group of compounds with common functional groups and divergent side chains. In this way, a researcher starting with three sets of 30 building blocks can create 27,000 (30 × 30 × 30) different compounds in two synthetic steps (Fig. 48-3). Whereas, in theory, one could create each compound individually in its own reaction well, in practice it is easier to synthesize the molecules on a solid support, such as a polystyrene bead. In a **parallel synthesis,** the beads are split so that thousands are reacted at once and then successively recombined and split to undergo successive reactions. Although this drastically decreases the number of reactions occurring (30 at a time instead of 27,000 at a time, in the example above), the challenge becomes sorting the beads to know which compound has been synthesized on each bead. Researchers have solved this problem by **tagging** the beads with a unique chemical code, such as a ribonucleotide sequence, during each reaction. To identify a bead that has yielded a successful hit, the tag is cleaved, amplified by standard methods, and sequenced. The code then reveals which reactions the bead has been exposed to, and consequently, the identity of the successful compound. Once a large library has been synthesized, it can then be screened in high throughput assays for activity, sometimes with the compounds still attached to the bead. The use of combinatorial chemistry and high-throughput screening is termed a **shotgun approach,** because researchers test a wide range of compounds against a single target. This approach can also be modified to look for a particular result by using "biased libraries" for different types of targets. For example, researchers have synthesized large libraries of compounds more likely to interact with G protein-coupled receptors, proteolytic enzymes, kinases, and ion channels, based on the structural characteristics present within each class of targets.

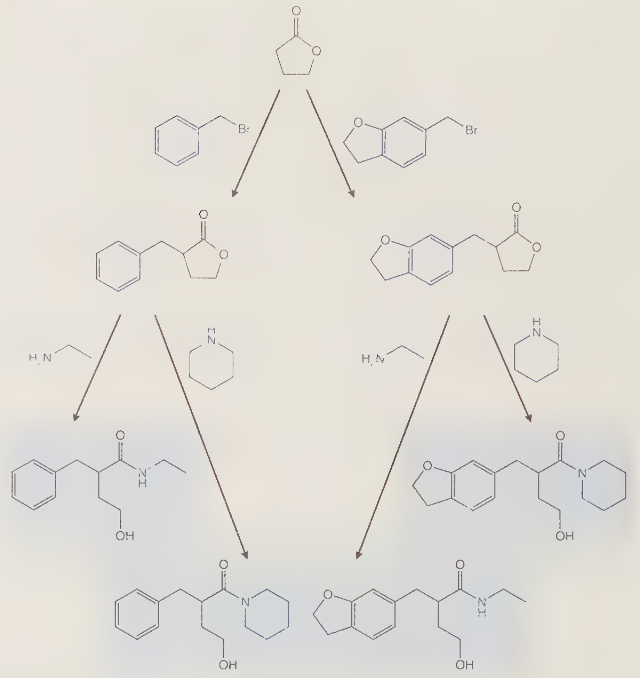


**Figure 48-3. Diversity through Combinatorial Chemistry.** Combinatorial chemistry uses simple building blocks to produce a complex library of compounds. In this example, the functionalized skeleton (black) has multiple sites of attachment. Two building blocks, shown in blue, combine with the functionalized skeleton to produce a wide variety of products. In this example, two different side groups for each of the two building blocks results in four ($2^2$) possible products. Combinatorial chemistry libraries use several building blocks, each with up to 20 or more different side groups, and can produce thousands of complex molecules using the same basic chemistry.

## Structure-Based Drug Design

Another target-centered approach is termed **structure-based drug design** or **rational drug design** because a drug candidate is discovered using the three-dimensional structure of a target obtained through **nuclear magnetic resonance** (NMR) or **X-ray crystallography.** Researchers start by obtaining a target's structure by X-ray diffraction, as Merck researchers did with HIV protease, or NMR spectroscopy of the target in solution. In theory, researchers can identify the active site within the structure of the target and use computers to study the shape of the active site. One could then design a molecule that is likely to fit in that space. More commonly, though, the target is cocrystallized with a substrate analogue, agonist, or antagonist ligand. This approach allows researchers to identify the active site. One can then modify the analogue using traditional synthetic manipulations to increase the drug's affinity, as was done in the case of ritonavir. Alternatively, researchers can refine the structure of the compound binding the target. In this way, the prototypic molecule is modified to improve its fit in the receptor's active site and hence increase its binding affinity (Fig. 48-1).

The advantages of this method are that the hits produced can be extremely potent, showing specificity in the nanomolar range. Moreover, only a limited number of candidates need to be tested, because the likelihood of a given compound binding is high. In addition, modification of the compound is easier than in other methods because researchers know which parts of the compound are critical for binding the active site. On the other hand, the modified compounds are often more difficult to synthesize because the molecular design demands specific functionality in specific locations. Thus, in a structure-based approach, fewer analogues are prepared (partly because of synthetic difficulty), but each analogue results in a higher improvement in activity compared to a structure-blind approach. The biggest challenge to this approach is obtaining a crystal structure of the target, which can be difficult, especially for membrane-bound proteins. Often, other methods of drug design yield hits long before the target can be crystallized. However, even if the initial hit results from another method, it can often be refined using structure-based drug design.

As the introductory case illustrated, rational drug design has been critical in the development of HIV protease inhibitors such as ritonavir. It has also been critical in the development of another class of antiviral drugs, the neuraminidase inhibitors (see Chapter 34). As structure-based drug design gains feasibility, more drugs will be produced using structural information about the target even if initial hits are discovered through other methods. For example, COX-2 selective inhibitors were developed to inhibit cyclooxygenase-2 selectively without interfering with the activity of COX-1 (see Chapter 40, Pharmacology of Eicosanoids). Although the structure of COX-2 was discovered later in the drug discovery process, verification of the drug selectivity and binding affinity was aided by structural studies of the drug and its target enzyme.

## PEPTIDES, PEPTIDOMIMETICS, AND MACROMOLECULES

The methods described above have focused on small molecules—compounds with a molecular weight under 500 Daltons. Most therapeutics fall into this category, largely because such compounds usually have high oral bioavailability, are easier to modify, and are generally cheaper to manufacture than larger ones. Increasingly, however, pharmaceutical companies are turning toward **peptides** or **peptidomimetic** compounds with molecular weights between 500 and 1,500 Daltons. Although these compounds are prone to intestinal and hepatic deactivation, coadministration of these larger drugs with small molecules that inhibit metabolism can increase oral bioavailability. For example, one of the reasons that the coadministration of ritonavir and lopinavir gained FDA approval (as described in the introductory case) is that lopinavir, when administered alone, is subject to high first-pass metabolism. Ritonavir inhibits intestinal and hepatic metabolism of lopinavir and thus increases plasma levels of lopinavir.

Pharmaceutical companies are also turning toward use of **macromolecules** such as **protein hormones, growth factors,** and **antibodies** (Table 48-2). Many of these macromolecules can be used as replacement agents in the treatment of diseases related to insufficiency of an endogenous compound, such as **insulin** for diabetes or **erythropoietin** for anemia. Natural or modified macromolecules are, however, increasingly used to modulate other physiologic processes, and engineered macromolecules, such as antibodies, are being used in the treatment of disease (see Chapter 49, Protein-Based and Gene Therapies). As the use of macromolecules has become more accepted, researchers have moved from finding relevant macromolecules in nature to engineering compounds

**TABLE 48-2** Examples of Macromolecular Therapies

| NAME | INDICATION | MOLECULAR CATEGORY | ORIGIN |
|---|---|---|---|
| Insulin | Diabetes | Hormone | Bacteria (recombinant human) |
| Erythropoietin | Anemia | Growth factor | Bacteria (recombinant human) |
| Heparin | Anticoagulant | Glycosaminoglycan | Porcine or bovine |
| Human growth hormone | Growth retardation | Hormone | Bacteria (recombinant human) |
| Parathyroid hormone | Osteoporosis | Hormone | Bacteria (recombinant human) |
| Trastuzumab | Cancer | Antibody | Chinese hamster ovary cell culture (humanized monoclonal antibody) |
| Streptokinase | Thrombolytic | Protein | Streptococcus |
| Antivenin | Snake bite | Antibody | Equine |

that interact or interfere with specific targets. Although these compounds must be administered parenterally, alternative delivery methods (pulmonary, nasal, buccal, transdermal, and oral) are also being studied.

## LEAD OPTIMIZATION

Drug discovery employs elements from many different approaches. Once a hit has been identified, much remains to be done to refine its characteristics and gather information about its physiochemical, biologic, and pharmacologic properties. This is the subject of the second part of this chapter, drug development. Most lead compounds have one or more characteristics (e.g., poor pharmacokinetics, difficulty in administration, toxicity) that make them poor candidates for clinical use. However, it is often possible to modify the structure of the molecule to overcome these deficiencies. As seen in the introductory case, precursors of ritonavir went through several modifications before a final compound was chosen to enter clinical trials. The process by which information gathered during the drug development process is used to modify the characteristics of a drug candidate is called **lead optimization.**

# DRUG DEVELOPMENT

**Preclinical drug development** refers to the research performed on a potential therapeutic agent before it enters clinical trials. Drug development can be divided into several broad categories. **Biological characterization** refers to research on how a potential drug interacts with biologic systems—including both its intended mechanism of action and its unintended side effects and toxicities—as well as the compound's distribution, pharmacokinetics, and metabolism. **Chemical characterization** refers to the chemical properties of the potential drug, including physical characteristics such as melting point, crystal form and solubility, as well as chemical characteristics such as purity and stability. The results of chemical characterization direct decisions regarding the shelf life of the drug and its method of administration and offer insights into possible modes of action and metabolic breakdown products. Finally, **chemical development** refers to three processes: **chemical synthesis** studies on how to make the drug efficiently; **commercial manufacturing** studies to produce the drug economically; and **drug formulation** studies about how to prepare the drug for administration. Most hits never reach the stage of clinical trials because they are found to lack effectiveness, have unfavorable toxicity or pharmacokinetic profiles, or are not amenable to large-scale and cost-effective manufacturing.

## BIOLOGICAL CHARACTERIZATION

One reason most hits never reach clinical trials is that rapid screening techniques are designed to be fast and simple. As such, these techniques rarely encompass the complex biological interactions that occur in vivo. Biological characterization is, thus, needed to help assess whether the molecule will likely work in humans (Table 48-3).

The goals of biological characterization are to gain insight into whether the molecule is likely to be efficacious and what its likely toxicities will be. Effectiveness is measured at the biochemical, cell, tissue, organ, and organism levels. If undesirable biological properties are found, the structure can be modified to improve its pharmacologic profile.

**Biochemical assays** evaluate potential mechanism of action at a molecular level. These tests include **receptor binding,** which measures both the binding affinity and selectivity for the target receptor. **Enzyme activity** measures the ability of a drug to inhibit the activity of an enzyme. Target selectivity is critically important in the design and testing of lead molecules.

In **cellular assays,** researchers aim to determine whether the lead molecule(s) acts appropriately in an environment that more closely approximates its in vivo use. For example, if the drug acts in the cytosol, it is essential to determine whether it can cross the plasma membrane. Toxicity is also measured by incubating the lead with a variety of tissues or cells, such as liver cells or extracts, to investigate its metabolic products, as well as how the molecule affects liver enzymes or interacts with other drugs. The ability of ritonavir to inhibit cytochrome P450 was found in such an assay. Complex patterns of gene expression are possible using gene array

TABLE 48-3 Information Obtained in Biological Characterization Studies

| TYPE OF ASSAY | EXPERIMENTAL TECHNIQUE | INFORMATION OBTAINED |
|---|---|---|
| Biochemical | Receptor binding | Receptor affinity/selectivity |
| | Enzyme inhibition | Enzyme inhibition/selectivity |
| | Metabolic processing | Metabolic products/metabolic inhibition |
| Cellular | Cell cultures | Cell penetration, receptor activity, transport |
| | Isolated tissue | Effects on specific tissue, possible toxicity |
| | Liver tissue (microsomes) | Metabolism, drug interactions |
| Systems/Whole animal | Rat, mouse | Toxicity, $LD_{50}$, organ-specific efficacy |
| | Dog, rabbit, pig, monkey | Pharmacokinetics/pharmacodynamics, bioavailability, toxicity |
| | Laboratory animal models for disease | Efficacy, toxicity, models for clinical trials |

chips capable of measuring mRNA levels for thousands of genes simultaneously.

Finally, at the highest level of complexity, the drug's effects on whole organisms are established. Ideally, **animal models** are used that mirror sufficient aspects of human pathophysiology for the target disease. For example, cancer chemotherapeutics can be tested in *nude* (T cell-deficient) mice that have been injected with human tumor cells. Pharmacokinetic properties are measured by observing blood levels of the drug over time. Drug biodistribution can be measured by administering radiolabeled drug and measuring radioactivity in organs. Using positron emission tomography, the biodistribution of radiolabeled drug can also be measured noninvasively in living animals. Metabolism is characterized by determining metabolic products in the animal's blood, urine, and feces. Most importantly, the drug's toxicity profile is measured by increasing the doses administered, both for acute and chronic exposure (see Chapter 51). Laboratory animal studies offer important insight into how to design clinical trials, by suggesting the variables that should be monitored and establishing possible endpoints and dosing regimens for clinical trials.

## CHEMICAL CHARACTERIZATION

The physical and chemical characteristics of a potential drug are critical for knowing how it can best be administered and stored (Table 48-4). The compound's **chemical structure** is commonly elucidated using **mass spectrometry,** which gives the compound's molecular weight; **elemental analysis,** which determines its atomic composition; **nuclear magnetic resonance (NMR),** which elucidates the types and patterns of chemical bonds present; and **X-ray crystallography,** which determines its three-dimensional structure. It is also important to distinguish among various isomers of the same compound, because biological activity is often isomer-dependent. For example, propranolol (see Chapter 23, Integrative Cardiovascular Pharmacology) is a mixture of *l* and *d* isomers, but only the *l* isomer acts as a β-adrenoceptor antagonist.

Chemists also characterize physical properties of the molecule, such as the $pK_A$ of an acidic or basic drug, which are used to aid in developing the formulation (see below). In addition, the drug's solubility is measured in a variety of solvents, especially water, to provide information on the molecule's bioavailability and possible metabolism. The **partition coefficient** describes how the molecule distributes between an aqueous solvent, analogous to blood, and a hydrophobic solvent, analogous to the plasma membrane. Finally, the compound's **stability** and **impurity profiles** must be determined.

## CHEMICAL SYNTHESIS AND MANUFACTURING

The amount of a drug candidate needed to run a simple screening assay is small—typically less than 1 mg. This is important, because synthesizing or isolating even small amounts of a compound can be expensive, at least until chemists can refine the synthesis. Once a lead is identified, gram quantities are needed to carry out the biological, toxicity, and chemical studies described above. Once a drug enters clinical trials, kilogram quantities are required and, if a drug is approved, plants will need to manufacture material on a scale needed to meet expected use. Quality and documentation of the specifications of the manufacturing process must be maintained throughout the scale-up (see Chapter 51).

### Chemical Synthesis

An effective chemical synthesis must satisfy several requirements. Ideally, it requires few synthetic steps. Each additional step in a chemical synthesis increases the possibilities of impurities, decreases the yield (the amount of material obtained at the end of the synthesis), and increases the costs. If multiple isomers of a compound are possible, a synthesis that produces only the target isomer is preferable. Finally, the synthesis should be amenable to scale-up.

Two techniques, **retrosynthetic analysis** and **convergent synthesis,** aid in the establishment of a good synthetic scheme. In a retrosynthetic analysis, key steps of a synthesis are developed by examining important structural elements in the final product and deciding how specific reactions could lead to the product (Fig 48-4). This procedure is performed iteratively so that a complex final molecule is reduced to simpler intermediates. The advantage of such an approach is that it greatly simplifies planning the synthesis of a complex product and readily leads to a convergent synthesis. In a convergent synthesis, in-

**TABLE 48-4** Information Obtained in Chemical Characterization Studies

| TYPE OF ASSAY | EXPERIMENTAL TECHNIQUE | CLINICAL IMPLICATIONS |
|---|---|---|
| Characterization, structure | NMR, IR, Mass spectrometry, X-ray crystallography | Isomeric purity, active compound |
| Solubility | Solubility in various solvents | Pharmacokinetics, metabolism, excretion, absorption, formulations |
| Partition coefficient | Octanol/water partition | Pharmacokinetics, metabolism, excretion, absorption, tissue distribution |
| Stability | Stability measurements under different conditions (heat, cold, humidity, light) | Shelf life, degradation products |
| Impurities | HPLC, GC, mass spectrometry | Possible adverse reactions from impurities, toxicology |

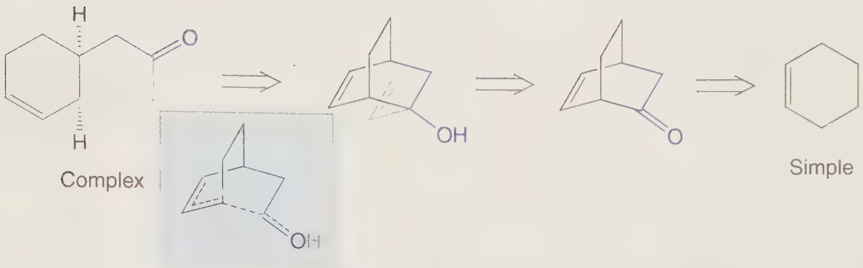


**Figure 48-4. Retrosynthetic Analysis of a Complex Molecule.** A retrosynthetic analysis of a complex molecule such as the bicyclic compound shown allows the identification of simple starting materials such as cyclohexadiene. Analysis of the structural element highlighted in blue demonstrates the creative process required to envision how a complex structure could be deconstructed into its component parts. The structure in a blue box illustrates the thinking required when deconstructing a molecule. These simple starting materials can then be combined in a series of steps to create the complex molecule. For simplicity, the synthetic details are not shown.

dividual parts of a molecule are synthesized separately, and the parts are assembled near the end of the synthesis (Fig 48-5). This increases the overall yield of the synthesis by reducing the number of linear steps required and allows each key component of the final product to be optimized individually. These two methods are complementary—often both are employed.

### Scale-up

For early development, the goal is to generate enough product to meet the demands of chemical and biological characterization, particularly for animal toxicology and formulation studies. As the scale of the need increases, the synthesis strategy must evolve. For example, a chemical synthesis often starts out using available raw material, which may include expensive specialty chemicals. However, as the scale of the synthesis increases, these reagents must be replaced with cheaper (and/or safer) alternatives. Although in an early synthetic scheme each intermediate is isolated, purified, and characterized to ensure that each sequential step of the synthesis is effective, as chemists gain more experience with the synthesis, multiple steps may be combined without isolating intermediates or purifying the products of each reaction, in a so-called **one-pot synthesis.**

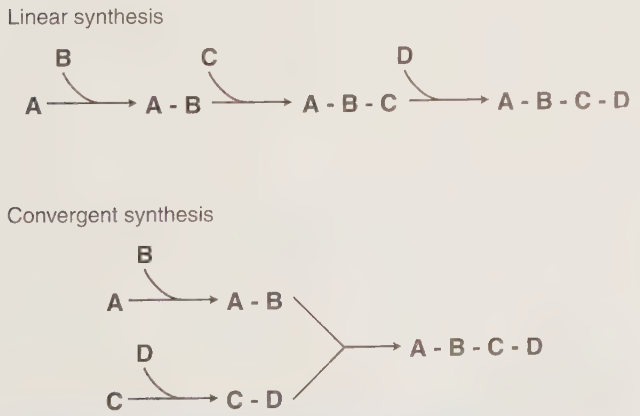


**Figure 48-5. Convergent Versus Linear Synthesis.** In a linear synthesis, each component is added sequentially. In a convergent synthesis, each component is assembled separately and then combined in the last step. The convergent synthesis approach generally results in higher yields. The arrows indicate sequential synthetic reactions.

### Macromolecule Development

Macromolecules such as insulin, growth hormone, and monoclonal antibodies are generally produced biologically, but must still be manufactured. For proteins, this usually involves expressing the gene in a bacterium, yeast, or a mammalian cell, and then growing the organism in large fermentation vats. The challenge for the manufacturer, then, is developing a system capable of producing the desired macromolecule and engineering methods of isolating the compound in pure form from the large mixture of metabolic products that often results.

### Manufacturing

Once the synthesis strategy of a drug candidate has been completed, process chemists must adapt the synthesis for large-scale commercial manufacturing. This process must be initiated before a drug is approved because the approval process requires that several batches of the drug be successfully manufactured, formulated (see below), and rigorously tested for quality and stability. A pharmaceutical company must also be ready to meet market demands immediately after approval, which requires that a manufacturing process be established before commercial launch.

The process chemist must ensure that the synthesis is safe and meets environmental regulations for emissions and disposal of water. This may preclude the use of certain solvents that are commonly used by synthetic chemists on a small scale.

## FORMULATION

Drugs must be in a form that can be administered to humans in a measured dose. The type of **formulation** used depends on the intended route of administration (Table 48-5). **Enteral formulations,** which include oral, sublingual, and rectal administrations, are absorbed through part of the digestive tract. **Parenteral formulations,** on the other hand, include intravenous, intramuscular, and subcutaneous injections, inhalants, topical formulations, and transdermal patches. The preferred route of administration is determined by many variables, including the drug's stability, its ease of absorption, first pass metabolism, bioavailability, and pharmacokinetics. Drugs that are relatively stable in the digestive tract, are not first-pass metabolized in the liver, have high oral bioavailability, and do not

**TABLE 48-5** Advantages and Disadvantages of Common Formulations

| FORMULATION | ADVANTAGES | DISADVANTAGES | EXAMPLES |
|---|---|---|---|
| **Enteral** | | | |
| Oral | Ease of administration | Slow absorption<br>First pass metabolism<br>Reduced bioavailability | Acetaminophen, pravastatin, oxycodone |
| Sublingual | Fast action<br>No first pass metabolism | Few drugs are absorbed | Nitroglycerin |
| Rectal | Fast action<br>No first pass metabolism | Uncomfortable | Morphine |
| **Parenteral** | | | |
| Intravenous | Fast action, high bioavailability, can control dose easily | Risk of infection, uncomfortable, must be administered by trained personnel | Lidocaine, morphine, tPA |
| Intramuscular | Sustained release possible | Uncomfortable, adverse reaction possible | Growth hormone, demerol |
| Subcutaneous | Slow action | Poor compliance | Insulin |
| Transdermal | Sustained release, no first pass metabolism | Poor absorption transdermally, slow acting | Nicotine (patch), estrogen |
| Inhalation | Large surface area for absorption | Inconvenience | Albuterol, glucocorticoids (asthma) |

require an immediate action are best administered orally. On the other hand, drugs that must be fast acting are better administered sublingually or intravenously. Macromolecules, which generally have little or no oral bioavailability, are normally administered via injection.

Most drugs are administered orally in either tablet or capsule form. In addition to the measured dose of the drug, most tablets contain **binders,** which keep the components together, and **stabilizers,** which enhance the drug's shelf life. For acid-sensitive drugs, it is often possible to coat the tablet with an **enteric coating** that is acid resistant but dissolves in the intestine. Formulation chemists can also manipulate the rate at which the tablet or capsule dissolves, thus enabling "long-acting" formulations whereby the drug is released slowly over the course of hours.

For intravenous administration, absorption profile and first pass metabolism are usually not issues. However, the drug must be dissolved in a vehicle, usually water. Moreover, the solution must be made isotonic with plasma by adding osmotically active compounds such as saline, dextrose, or mannitol, so that the solution does not cause hemolysis. The solution to be injected must also be sterile. Finally, a drug is often less stable in solution than as a solid, so formulation chemists must test its stability in solution. If it is unstable, the drug may be prepared as a **lyophilized powder** that can be dissolved in water or buffer immediately before administration.

## Conclusion and Future Directions

The discovery and development of new drugs is a complex process that often requires 10 or more years and hundreds of millions of dollars. Researchers start by searching for a biologically active compound. This may involve a drug-centered approach or a target-centered approach. Once a potential candidate molecule has been discovered, it must be characterized chemically and biologically. Its synthesis and manufacturing processes must be devised, and its method of administration determined. If a drug has favorable chemical and biological characteristics, and shows a likelihood of effectiveness in animal models of disease with few adverse effects, it may be tested in humans (see Chapter 51).

New pharmacologic targets are being found by gene sequencing, by analysis of genetic factors that predispose to disease, and by gene knockout experiments in laboratory mice. New methods to discover compounds that interact with these targets are becoming available. It is possible to target proteins that enable the expression of genes rather than the gene products themselves. Information about polymorphisms within genes may enable the products of specific, mutant genes to be the targets of new drugs (see Chapter 52, Pharmacogenomics).

## Suggested Readings

Burke MD, Schreiber S. A planning strategy for diversity-oriented synthesis. Angew Chem Int Ed 2004;43:46–58. (*Tools synthetic organic chemists use to synthesize a specific target or a large library.*)

Drews J. Drug discovery: a historical perspective. Science 2000; 287:1960–1964. (*Historical description of the main methods of drug discovery.*)

Levine RR. Pharmacology, Drug Actions and Reaction. 6th Ed. New York: Parthenon Publishing Group, 2000. (*Excellent explanation of how new drugs are found, as well as a description of the development process through clinical development.*)

Rademann J, Günther J. Integrating combinatorial synthesis and bioassays. Science 2000;287:1947–1948. (*Novel techniques for screening large libraries of compounds.*)

# 49

# Protein-Based and Gene Therapies

Benjamin Leader, J. Wesley Ulm, Xandra O. Breakefield, and David E. Golan

**Protein-Based Therapy**
- Proteins as Medicines: An Introduction
- Uses of Proteins in Medicine
  - *Group I: Protein Therapeutics with Enzymatic or Regulatory Activity*
  - *Group II: Protein Therapeutics with Special Targeting Activity*
  - *Group III: Protein Vaccines*
  - *Group IV: Protein Diagnostics*
- Challenges of Protein-Based Therapeutics

**Gene Therapy**
- Introduction
- Why Gene Therapy?
- Classes of Disease Targets
  - *Diseases Attributable to Simple Recessive Mendelian, X-linked, Triplet-Repeat, or Mitochondrial Inheritance*
  - *Protein Aggregation Diseases*
  - *Neoplastic Diseases*
  - *Cardiovascular Diseases*
  - *Infectious Diseases*
- Requirements for Safe and Effective Gene Therapy
- Viral Gene Therapy Vectors: Applications and Limitations
  - *Retroviruses*
  - *Adenoviruses*
  - *Adeno-Associated Viruses (AAVs), Herpesviruses, Epstein–Barr Virus*
  - *Antisense, RNA and RNA Interference*

**Conclusion and Future Directions**

**Suggested Readings**

## PROTEIN-BASED THERAPY

### PROTEINS AS MEDICINES: AN INTRODUCTION

Proteins, nucleic acids, carbohydrates, and lipids comprise the four classes of large molecules in the human body. Of these four classes, proteins play the most dynamic and active role, displaying immense diversity of function. For example, proteins known as enzymes catalyze almost every biochemical reaction. Proteins can also form receptors and channels in membranes, provide intracellular and extracellular scaffolding support, and transport molecules within a cell or from one part of the body to another. A specific gene encodes the blueprint for the construction of each protein. Over time, evolution has selected for genes encoding a vast number of proteins, each with a specific role, that control the function of the body. It is currently estimated that there are 30,000 different genes in the human genome, which theoretically means that there are 30,000 different proteins. However, alternative splicing of genes and postranslational modification of proteins (e.g., by cleavage, phosphorylation, and glycosylation) causes the number of functionally distinct proteins to be much higher. Disease may result when one of these proteins is defective or present in an abnormally high or low concentration.

It is not surprising that medicine has begun to harness protein-based therapies to alleviate human suffering. Protein-based therapies have several advantages over small-molecule therapeutic agents. First, proteins often serve a highly specific and complex set of functions that cannot be mimicked by simple chemical compounds. Second, because the action of proteins is highly specific, there is often less potential for protein-based drugs to interfere with normal biological processes and cause side effects. Third, because the body naturally produces many of the proteins that are used as therapies, such drugs are often well tolerated and are less likely to elicit immune responses. (One exception to this generalization is a therapeutic protein used to replace a factor that has been missing since birth. For example, anti-factor VIII antibodies ("inhibitors") can develop in patients with severe hemophilia who are treated with recombinant human factor VIII.) Fourth, for diseases in which a gene is mutated or deleted, protein-based therapies can provide effective replacement treatment without the need for gene therapy, which is not currently available for most genetic disorders. Finally, because proteins are unique in form and function, pharmaceutical companies are able to obtain far-reaching patent protection for protein therapeutics. This makes proteins attractive from a financial perspective as compared to chemical compounds, which can frequently be copied with slight modifications that circumvent patent protection.

**Insulin** therapy for diabetes mellitus is perhaps the best and most illustrative example of how a protein has come to be used therapeutically in medicine. Diabetes mellitus type I (DM-I), formerly known as insulin-dependent diabetes mellitus, is a disease in which the body produces insufficient quantities of the protein hormone insulin. Insulin signals cells to perform a number of functions related to glucose homeostasis and intermediary metabolism (see Chapter 28, Pharmacology of the Endocrine Pancreas). Before the advent of insulin therapy, DM-I caused severe wasting and was usually a fatal disease. In 1922, insulin was first purified from bovine and porcine pancreas and used therapeutically as a life-saving daily injection. The immediate success of this work was apparent, resulting in a Nobel Prize only one year later in 1923. However, some patients developed antibodies to the insulin injections because the animal insulin is not identical in sequence to human insulin and is recognized as a foreign protein. In addition, purifying insulin from animal pancreas was expensive.

Once the human gene for insulin had been isolated, recombinant DNA technology could be used to produce the human form of the protein. This technology has allowed the human insulin gene to be engineered into circular strands of DNA or plasmid expression vectors. After insertion into bacteria, these expression vectors contain DNA sequences that cause high expression of the human insulin gene. The bacteria are grown in large quantities, and the human insulin is purified from the bacterial culture. This results in the production of large amounts of insulin that is inexpensive, of low immunogenicity, and free from other animal pancreatic substances. Recombinant insulin was the first commercially available recombinant protein drug; it was approved by the United States Food and Drug Administration (FDA) in 1982. It has been the major therapy for DM-I ever since.

Protein therapies today are produced recombinantly (like insulin), purified from discarded human tissues, or produced in other organisms. For example, **streptokinase,** used clinically to disrupt life-threatening blood clots in emergent conditions such as pulmonary embolism and acute myocardial infarction, is purified from Group C β-hemolytic streptococci (see Chapter 21, Pharmacology of Hemostasis and Thrombosis). "Lactose-intolerant" patients, who have a deficiency in the enzyme that cleaves lactose to galactose and glucose, are treated with **lactase** produced by the fungus *Aspergillus oryzae.* Thus, a variety of organisms are used as sources for purification of protein-based therapies.

Genetic engineering and biotechnology methods have allowed proteins to be produced using recombinant DNA. The development of this technology has been impressively rapid. The structure of DNA was first reported in 1953, and most of the genetic code was deciphered by 1965. The first successful cloning experiments were reported in 1973. In 1976, only 23 years after the structure of DNA had been elucidated, **somatostatin,** a 14 amino acid hormone, was the first human protein to be produced by recombinant DNA technology. Shortly thereafter, recombinant human insulin became commercially available. Since then, the process of producing recombinant proteins has been steadily improved. Once a gene of interest has been identified and isolated, it can be "recombined" with other genes that allow expression and large-scale production of the "gene product" (i.e., protein) in a biological system of choice.

A wide variety of systems are used today to produce recombinant proteins, including bacteria, yeast, insect cells, mammalian cells, transgenic animals, and plants. The system of choice can be dictated by the cost of production or the modifications of the protein (e.g., glycosylation, phosphorylation, or proteolytic cleavage) that are required for biological activity. For example, bacteria do not perform glycosylation reactions. Each of the other biological production systems listed above produces a different type of glycosylation, which can affect the activity, half-life, or immunogenicity of the recombinant protein in the body.

Recombinantly produced proteins have all the advantages of standard purified proteins, with the following additional benefits. First, transcription and translation of the exact human gene leads to a higher specific activity of the protein and a decreased chance of immunologic rejection by the patient, as illustrated above using the example of bovine insulin compared to recombinant human insulin. Second, recombinant proteins are often produced more efficiently and inexpensively, and in potentially limitless quantity. One striking example is found in the protein-based therapy for Gaucher's disease, a chronic congenital disorder of lipid metabolism caused by a deficiency of the enzyme beta-glucocerebrosidase. Most patients with this disease have an enlarged liver and spleen, increased skin pigmentation, and painful bone lesions. Although patients can be treated with purified **beta-glucocerebrosidase** protein, this treatment requires purification of protein from 50,000 human placentas per patient per year. This requirement obviously places a practical limit on the amount of purified protein available for patients with the disease. Although still extremely expensive, a recombinant form of beta-glucocerebrosidase is now available. The recombinant protein not only provides sufficient quantities to treat many more patients with the disease, but also eliminates the risk of transmissible (viral) diseases associated with purifying the protein from human placentas. This illustrates the third benefit of recombinant proteins over purified proteins, which is the reduction of exposure to animal or human diseases. A fourth advantage is that recombinant technology allows the modification of a protein to improve function or specificity. Again, recombinant beta-glucocerebrosidase provides an interesting example. When this protein is made recombinantly, a change of amino acid arginine-495 to histidine allows the addition of mannose residues to the protein. Mannose is recognized by endocytic carbohydrate receptors on macrophages and many other cell types, allowing the enzyme to enter these cells more efficiently and to cleave the pathologic lipid that has accumulated. This results in an improved therapeutic outcome.

Protein-based therapies have been used for decades in medicine, with many positive results. The past 20 years, however, have seen a remarkable expansion of proteins in the pharmacologic armamentarium used by physicians to treat human disease. More than 40 different recombinant proteins are now approved for clinical use by the FDA, and many more are in development. Protein-based therapies are, and will continue to be, a mainstay in treating human disease.

## USES OF PROTEINS IN MEDICINE

Proteins from both recombinant and purified sources are employed in a wide variety of medical applications, ranging from alleviating mild digestive ailments to correcting lethal protein deficiencies to diagnosing infectious diseases. These proteins can be categorized according to their mechanism of action, allowing the creation of a comprehensive structure for facile understanding of the therapeutic uses of the proteins (Table 49-1; see also an expanded version of this table at the end of the chapter). A few of these proteins can be used clinically in more than one way; such proteins may, therefore, fall into more than one category.

### Group I: Protein Therapeutics with Enzymatic or Regulatory Activity

This category involves the classic paradigm in which a specific endogenous protein is deficient, and the deficit is then remedied by therapy with exogenous protein. *Group Ia* proteins are used to replace a particular activity in cases of protein deficiency or abnormal protein production. The classic example, as mentioned above, is the use of insulin for the treatment of diabetes. In diabetes mellitus type I, the β-cells in pancreatic islets of Langerhans are destroyed, leading to a deficiency of insulin production. Normal insulin levels are achieved instead by treatment with recombinant human insulin produced in *E. coli*.

Another important example is the treatment of cystic fibrosis, the most common lethal genetic disorder. In this disease, defects in the chloride channel encoded by the CFTR gene lead to abnormally thick secretions, which can (among other effects) block pancreatic enzymes from traveling down the pancreatic duct into the duodenum. This prevents food from being properly digested and results in malnutrition. Patients with cystic fibrosis are often treated with a combination of **pancreatic enzymes** isolated from pigs—including lipases, amylases, and proteases—that allow for digestion of lipids, sugars, and proteins. Patients who have had their pancreas removed or who suffer from chronic pancreatitis can benefit from this therapy as well.

**TABLE 49-1** A Functional Classification of Protein-Based Therapies

**Group I: Protein Therapeutics with Enzymatic or Regulatory Activity**
- Ia: Replaces a protein that is deficient or abnormal
- Ib: Augments an existing pathway
- Ic: Provides a novel function or activity

**Group II: Protein Therapeutics with Special Targeting Activity**
- IIa: Interferes with a molecule or organism by binding to it and thereby blocking its function or targeting it for degradation
- IIb: Stimulates a signaling pathway
- IIc: Delivers other compounds or proteins

**Group III: Protein Vaccines**
- IIIa: Protects against a deleterious foreign agent
- IIIb: Treats an autoimmune disease
- IIIc: Treats cancer

**Group IV: Protein Diagnostics**

It may sometimes be desirable to augment the activity of a particular plasma protein that is present in normal amounts. *Group Ib* proteins are administered to enhance the magnitude or timing of a particular protein activity. Recombinant proteins in this category can be immensely successful in treating hematopoietic defects. The most prominent example is recombinant **erythropoietin,** a protein hormone secreted by the kidney that stimulates red blood cell (erythrocyte) production in the bone marrow. In patients with anemia caused by chemotherapy, myelodysplastic syndrome, and other conditions, recombinant erythropoietin is used to increase erythrocyte production and, thereby, ameliorate the anemia. In patients with renal failure, whose levels of endogenous erythropoietin are below normal, recombinant protein is administered to correct this deficiency.

In vitro fertilization (IVF) provides another example of the therapeutic use of a *Group Ib* protein. In attempting to maximize the number of available oocytes for IVF to treat certain forms of female infertility, ovarian follicle maturation can be enhanced by treatment with recombinant **follicle stimulating hormone** (FSH). Increased levels of FSH are normally produced by the anterior pituitary gland prior to and just after ovulation. These high levels of FSH can be further augmented by using the recombinant form of the protein, leading to maturation of an increased number of follicles.

A third example is the use of recombinant **tissue plaminogen activator** (tPA) to treat a life-threatening blood clot, such as a coronary artery occlusion or pulmonary embolism. Endogenous tPA is secreted by the endothelial cells that line blood vessels. The secreted tPA cleaves plasminogen to plasmin, which then degrades fibrin and thereby lyses fibrin-based clots (see Chapter 21). Although endogenous tPA may be present at normal or even increased levels near the site of a blood clot, administration of relatively large amounts of exogenous tPA may be required to disrupt these clots. As illustrated by these three examples, modern medicine has not only identified important human proteins and their functions, but can now also modify protein activity levels when the human body is unable to do so optimally.

Occasionally, the activity of a particular protein is desirable even though the body does not normally express that activity. *Group Ic* proteins contain examples of this paradigm, including both foreign proteins with novel functions and endogenous proteins that act at a novel time or place in the body. **Papain,** for example, is a protease purified from the *Carica papaya* fruit. This protein is used therapeutically to degrade proteinaceous debris in wounds. The protease-mediated debridement or removal of necrotic tissue is helpful in the treatment of pressure ulcers, postoperative wounds, carbuncles, and other types of wounds. Recombinant human **deoxyribonuclease I** also has an interesting novel use. Normally found inside human cells, this recombinant enzyme can be used to degrade the DNA left over from dying neutrophils in the respiratory tracts of patients with cystic fibrosis. Such DNA could otherwise form mucous plugs that obstruct the respiratory tract and lead to pulmonary fibrosis, bronchiectasis, and recurrent pneumonias. Thus, recombinant protein technology has allowed modern medicine to employ a normally intracellular enzyme in a novel extracellular environment.

## Group II: Protein Therapeutics with Special Targeting Activity

The exquisite binding specificity of **monoclonal antibodies** can be exploited in a variety of ways using recombinant DNA technology. Many *Group IIa* proteins, known as **immunoadhesins,** use the antigen recognition sites of immunoglobulin molecules to guide the body's immune system to destroy specifically targeted molecules or cells. Other immunoadhesins neutralize molecules by simple physical occupation of a functionally important region of the molecule. Immunoadhesins combine the antigen recognition sites of known immunoglobulins with a domain of the same or a related immunoglobulin known as the Fc region. Fc regions can target a soluble molecule for destruction because cells of the immune system can recognize the Fc region, endocytose the attached molecule, and break down the molecule chemically and enzymatically. When attached to specifically recognized molecules on the surface of a cell, the Fc region can target the cell for destruction by the immune system. Cell killing can be mediated by macrophages, by other immune cells, or by complement fixation. The latter is a process in which the Fc region of an immunoglobulin bound to the cell surface recruits certain plasma proteins (called complement proteins) that create holes in the cell membrane.

One example of a *Group IIa* protein is **infliximab.** This recombinantly produced monoclonal antibody binds tumor necrosis factor alpha (TNF-$\alpha$), a cytokine that can stimulate increased activity of the immune system. Infliximab is used to neutralize the action of TNF-$\alpha$ in inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease (see Chapter 43, Pharmacology of Immunosuppression). Another example of the use of a *Group IIa* protein is the prevention of severe infection by respiratory syncytial virus, which is one of the leading causes of hospital admissions for pediatric respiratory illness. High-risk patients are given **palivizumab,** a recombinantly produced monoclonal antibody that binds to a particular molecule on the virus, called the F protein, leading to increased immune-mediated clearance of virus from the body.

Many processes in the body are modulated by cell surface receptors that are activated upon binding of their cognate ligands (see Chapter 1, Drug–Receptor Interactions). By binding to such receptors, *Group IIb* targeting proteins can activate cell signaling pathways and profoundly affect cell function. Outcomes range from cell death (through the induction of apoptosis), to downregulation of cell division, to increased cell proliferation. Although it is currently difficult to prove that a particular target-binding protein mediates an in vivo effect through the modulation of a particular signaling pathway, in vitro evidence suggests that this type of modulation is the mechanism of action of certain therapeutic proteins. For example, the treatment of certain breast cancers, in which the malignant cells express the Her2/Neu cell surface receptor, is enhanced by the addition of **trastuzumab** (an anti-Her2/Neu monoclonal antibody) to the therapeutic regimen. Although trastuzumab contains an Fc region, it is unlikely that simple targeting of the immune system to breast cancer cells by trastuzumab is sufficient to mediate cell killing. This is because many other monoclonal antibodies, with similar abilities to target breast cancer cells, have failed to show efficacy in vivo. Rather, because the anti-Her2/Neu antibody has been shown in vitro to induce intracellular signaling events that control the growth of breast cancer cells, it is likely that receptor-mediated signaling accounts for the efficacy of trastuzumab in vivo. Although there are currently not many examples of *Group IIb* proteins in clinical use, more are in development and will likely be available in the coming years.

One of the great challenges in pharmacotherapy is the selective delivery of therapeutic chemicals and proteins to the correct location in the body. The human body normally uses proteins to achieve specialized transport and delivery of molecules. An active area of current research is focused on understanding the principles of protein-based, targeted delivery of molecules, so that these principles can be applied to modern pharmacotherapy. Although there are currently no FDA-approved examples of the *Group IIc* category—proteins that enable drug delivery to a specific site—approved for clinical use, there are interesting developments in progress that illustrate where the field may be headed. For example, Herpes simplex virus produces a protein, VP22, that enters human cells. VP22 has been used in vitro to deliver proteins or other compounds to the nucleus. For example, VP22 was used to target the tumor suppressor protein p53 to cultured osteosarcoma cells that lacked the p53 gene (and hence the protein). Reintroduction of p53 led to apoptosis of the cells. It is currently thought that a novel and effective therapy for certain forms of cancer could employ protein-based targeting of the p53 gene.

Another challenging area of research involves the delivery of proteins and other macromolecules to the central nervous system (CNS). This is currently virtually impossible because of the highly selective blood–brain barrier (BBB). Animal experiments have demonstrated, however, that fusion proteins combining a therapeutic protein with a protein that naturally has specific access through the BBB can allow successful delivery of the therapeutic protein to the CNS. For example, a fragment of the tetanus toxin protein that naturally crosses the BBB has been shown in animal experiments to deliver the enzyme superoxide dismutase to the CNS. This type of therapeutic could be used to treat neurologic disorders such as amyotrophic lateral sclerosis, in which CNS levels of superoxide dismutase are abnormally low. Exciting prospects also exist for the treatment of other disorders of the CNS in which levels of a particular protein are abnormal.

## Group III: Protein Vaccines

As recombinant DNA technology was being developed, great strides were also being made in understanding the molecular mechanisms that allow the human immune system to protect the body against infectious diseases and cancer. Armed with this new understanding, *Group III* proteins have been successfully applied as prophylactic or therapeutic vaccines. For humans to develop effective immunity against foreign organisms or cancer cells, immune cells such as helper T cells must be activated. Immune cell activation is mediated by antigen-presenting cells, which display on their surface specific oligopeptides that are derived from proteins found in foreign organisms or cancer cells. Vaccination against organisms such as polio or measles has most often been achieved by injecting heat-killed or attenuated forms of these pathogens. Unfortunately, these methods have involved a certain amount of unavoidable risk of infection or adverse reaction. By specifically injecting the appropriate immunogenic (but nonpathogenic) protein components of a microorganism, vaccines can be created that provide immunity in an individual without exposing the individual to the risks of infection.

*Group IIIa* proteins are used to generate protection against infectious diseases or toxins. One very successful example is the **Hepatitis B vaccine.** This vaccine was created by producing recombinant HBsAg protein, a noninfectious protein of the Hepatitis B virus. When this protein is repeatedly injected into immunocompetent humans, significant immunity results in the large majority of individuals. In addition to generating protection against foreign invaders, recombinant proteins can induce protection against an overactive immune system that attacks its own body or "self." One theory is that administering large amounts of this self-protein causes the body's immune system to develop tolerance to that protein by eliminating or deactivating cells that react against the self-protein. *Group IIIb* proteins are used to treat patients with disorders that arise from this type of autoimmune phenomenon. One example is the use of **glutiramer acetate,** a short protein of 4 amino acids. When administered to patients, this protein can improve the symptoms of certain forms of multiple sclerosis, an autoimmune disorder that targets the nervous system.

*Group IIIc* proteins are used to vaccinate against cancer. Although there are currently no FDA-approved recombinant anticancer vaccines, there are promising clinical trials that employ patient-specific cancer vaccines. For example, a vaccine for B-cell non-Hodgkin's lymphoma uses transgenic tobacco plants (*Nicotiana benthamiana*). Each patient with this type of lymphoma has a malignant proliferation of an antibody-producing B-cell that displays a unique antibody on its surface. By subcloning the idiotype region of this tumor-specific antibody and producing the region recombinantly in tobacco plants, a tumor-specific antigen is produced that can be used to vaccinate a patient. (This is somewhat ironic, in that the immune system is being vaccinated to protect against an antibody-producing cancer.) This process requires only 6–8 weeks from the biopsy of the lymphoma to a ready-to-use, patient-specific vaccine. As the genomes of infectious organisms and the nature of autoimmune diseases and cancer are more fully elucidated, more recombinant proteins will undoubtedly be developed for use as vaccines.

## Group IV: Protein Diagnostics

Although *Group IV* proteins are not used to treat disease, purified and recombinant proteins used for medical diagnostics bear mentioning here because they are invaluable in the decision-making process that precedes the treatment and management of many diseases. A classic example is the **purified protein derivative** (**PPD**) test, which determines whether or not an individual has been exposed to antigens from *Mycobacterium tuberculosis*. In this example, a noninfectious protein component of the organism is injected under the skin of an immunocompetent patient. An active immune reaction, characterized by redness and induration at the injection site, is interpreted as evidence that the patient has been previously infected by *M. tuberculosis* or exposed to the antigens of this organism. Another example illustrating the use of proteins for diagnosis is provided by recombinant **growth hormone releasing hormone** (GHRH). This protein stimulates somatotroph cells of the anterior pituitary gland to secrete growth hormone. Used as a diagnostic, GHRH can help to determine whether pituitary growth hormone secretion is defective in patients with clinical signs of growth hormone deficiency (see Chapter 24, Pharmacology of the Hypothalamus and Pituitary Gland). A final example of the use of proteins in diagnosis involves the natural and recombinant human immunodeficiency virus (HIV) antigens that are essential components of common screening (enzyme immunoassay) and confirmatory (western blot) tests for HIV infection. In these tests, the antigens serve as "bait" for specific antibodies to HIV *gag, pol,* and *env* gene products that have been elicited in the course of infection.

# CHALLENGES OF PROTEIN-BASED THERAPEUTICS

Although there are now many examples in which proteins have been used therapeutically in clinical medicine, the attempted protein therapies that have failed far outnumber the successes. The production of biologically active proteins can be difficult for a number of reasons. *First,* drug delivery (pharmacokinetics) provides one of the greatest challenges to successful protein therapy. Proteins are large molecules with both hydrophilic and hydrophobic properties that can make entry into cells and other compartments in the body difficult. Protein solubility, stability, route of administration, and distribution are all factors that can hinder the successful application of protein therapy. The stability of the protein inside the human body is another important pharmacokinetic issue. The half-life of a therapeutic protein can be drastically affected by proteases, protein-modifying chemicals, or other clearance mechanisms. *Second,* recombinant proteins must be synthesized in a genetically engineered cell type for large-scale production. Although bacteria and yeast are generally easy to culture, certain mammalian cell types can be more difficult and more costly to culture. Without a reasonable system for the production of large amounts of a protein, the protein's commercial viability is limited. *Third,* for a protein to be physiologically active, post-translational modifications, such as glycosylation, phosphorylation, and proteolytic cleavage, are often required. These requirements may dictate the use of specific cell types that are capable of expressing and modifying the protein appropriately. *Fourth,* the body may mount its own immune response against the therapeutic protein. In some cases, this immune response can neutralize the protein, and can even cause a harmful reaction in the patient. Until recently, the widespread clinical application of monoclonal antibodies has been limited by the rapid induction of immune responses against this class of therapeutic proteins. With the advent of methods to develop "humanized" antibodies—in which portions of the antibody that are not critical for antigen-binding specificity are replaced with human immunoglobulin sequences that confer stability and biologic activity on the protein but do not provoke an "anti-antibody" response–this limitation has been overcome, at least in some cases. *Last,* but certainly not least, the costs involved in developing protein therapies can be prohibitive. Because most protein-based therapeutics are produced by companies that must remain financially solvent, cost can often be the crucial issue. For example, switching to recombinant methodology from laborious purification of placentally-derived protein has allowed the production of sufficient β-glucocerebrosidase to treat Gaucher's disease in many patients. Even so, the cost of the recombinant protein currently ranges from $85,000 to $350,000 per patient per year, which prevents some patients from receiving the necessary therapy.

# GENE THERAPY

## INTRODUCTION

Gene therapy is a novel pharmacologic approach in which the drug is supplied in the form of a nucleic acid—DNA, RNA, or some modification or combination thereof. The origin of gene therapy lies in the watershed advancements of two fields, particularly in the 1970s and 1980s: recombinant DNA technology, which enables the precise manipulation and amplification of defined DNA segments; and virology, in which progress in the basic science of viral life cycles and replication has allowed for the exploitation of viral particles as vectors to ferry therapeutic DNA or RNA into cells. Although many obstacles remain to be overcome before gene therapy can enter the mainstream of treatment and prevention of human disease, the technology holds great promise. Effective and safe gene therapy would bring pharmacology to the core of the central dogma in biology, treating disease at the level of the nucleic acid polymers that control the fundamental processes of normal physiology and pathophysiology.

## WHY GENE THERAPY?

From a pharmacologic perspective, gene therapy presents an attractive option because its very nature is conducive to a high therapeutic index, at least at the cellular level. A DNA or RNA sequence prefabricated to target a particular disease locus constitutes, in principle, a highly specific and minimally toxic therapeutic. (As discussed below, the viral vectors currently used in most gene therapy protocols unfortunately introduce a degree of nonspecificity and toxicity, often in the form of an immune response.) Gene therapy also affords the prospect of long-term effectiveness from a small number of interventions, enabling physicians to treat efficiently many classes of disease that would otherwise be resistant to therapy. Heritable loss-of-function genetic diseases such as cystic fibrosis (CF), the hemophilias, sickle-cell anemia, Duchenne muscular dystrophy, and many others represent the classic targets for gene therapy. A gene therapy protocol providing a stable supply of a missing or damaged protein could obviate the need for the chronic, expensive, and often only partially effective (e.g., hemophilia, the thalassemias) or palliative (e.g., cystic fibrosis, Tay-Sachs disease, Lesch–Nyhan syndrome) treatments that currently exist. In practice, most protocols recently have focused on cancer, heart failure, and angiogenesis, but recessive genetic diseases still represent the canonical targets for which gene therapy was originally conceived.

Finally, gene therapy provides a flexible treatment strategy that could be readily adapted to treat novel pathogens and even overcome resistance. Modern medicine, particularly the antimicrobial and anticancer chemotherapeutic drugs introduced after World War II, has in some respects become a victim of its own success. Such treatment regimens frequently become case studies in cellular microevolution, as the drugs ultimately select for targets that are resistant to their mode of action (see Chapter 38, Principles of Combination Chemotherapy). This challenge has repeatedly foiled many a promising new cancer treatment: a novel drug seems to clear the tumor from a patient's body, only to have a relapse months or years later due to the outgrowth of a few cells expressing a multidrug-resistance membrane pump or an overamplified or mutated protein target. It is here that a gene therapy scheme could demonstrate great benefit. If a drug target (e.g., a pathogenic bacterium or a relapsing tumor) were to acquire a resistance phenotype against a small molecule therapeutic, it would be a long and time-consuming process to generate and screen new small molecules to adapt to the change, even with modern improvements in computer modeling and robotic technologies. A linear DNA sequence, in contrast, could be rapidly and reliably altered to target the new gain-of-resistance mutation. In a novel paradigm of treatment, gene therapy remedies could be tailored to each patient and dynamically adapted to respond to resistance as it arises.

The next sections discuss the various gene therapy strategies, organized according to classes of disease targets, requirements for safe and effective treatment, and viral gene vectors currently under development.

## CLASSES OF DISEASE TARGETS

To which areas of human disease could a viable gene therapy protocol be applied to the greatest advantage? Although this answer can only be conjectured at the present time, current proposals for clinical trials and topic reviews tend to focus on five categories: diseases attributable to simple recessive mendelian, X-linked, triplet-repeat, or mitochondrial inheritance; protein aggregation diseases; neoplastic diseases; cardiovascular diseases; and infectious diseases.

### Diseases Attributable to Simple Recessive Mendelian, X-linked, Triplet-Repeat, or Mitochondrial Inheritance

A number of human diseases can be traced to mono- or diallelic mutations in a single gene that is crucial for homeostasis. A large class of disorders is caused by homozygous recessive mutations that cause a loss of function in a critical protein. Such anomalies are notoriously difficult to treat with conventional remedies. Although a deficiency in a circulating molecule such as insulin (diabetes mellitus type I) or factor IX (hemophilia B) can be at least partially ameliorated by exogenous administration of the deficient protein, a loss of function in a cognate receptor, intracellular enzyme, or structural protein is typically unresponsive to such therapy. Unless the mutation can be circumvented, this loss of function may present an intractable danger to the patient's health. The ΔF508 mutation in cystic fibrosis (CF)—caused by the loss of a single phenylalanine codon in the CFTR gene on both chromosomes—renders an otherwise functional chloride transporter incapable of reaching the plasma membrane of epithelial cells, instead causing its sequestration in the endoplasmic reticulum and its subsequent degradation by proteasomes. This homozygous deletion (and others like it) affects critical cellular functions such as water transport, and ultimately leads to the fatal sequelae of CF. Similarly, familial hypercholesterolemia (FHC) is caused by mutations in the gene coding for the cell surface low-density lipoprotein (LDL) receptor; decreased or absent receptor expression prevents cellular uptake of LDL and leads to high levels of plasma LDL that cause accelerated atherosclerosis (see Chapter 22, Pharmacology of Cholesterol and Lipoprotein Metabolism). A litany of enzyme deficiencies—for example, Tay-Sachs disease (hexosaminidase A); Gaucher's disease (glucocerebrosidase); ataxia-telangiectasia, Bloom's syndrome, and Fanconi's anemia (DNA-repair enzymes); immune system disorders such as adenosine deaminase (ADA) deficiency and chronic granulomatous disease (NADPH oxidase)—result in usually fatal and (with the exception of Gaucher's) incurable illnesses in affected patients. Mutations in intracellular structural proteins (muscular dystrophy), membrane components (myelin in Type Ia Charcot-Marie-Tooth syndrome), and specialized functional proteins (hemoglobin in sickle cell anemia and thalassemia) also pose detrimental or even grave threats to the health of affected individuals.

Most of the above are examples of recessive or X-linked diseases. Some disorders transmit in families according to autosomal dominant patterns. Neurofibromatosis 1 (consequent to germline monoallelic loss of neurofibromin, a GTPase-activating protein) and Li-Fraumeni syndrome (consequent to germline monoallelic loss of one p53 allele) are both recessive at the cellular level, because they require two hits against a tumor-suppressor protein to alter a given cell. However, if one allele is mutated in the germline, then stochastic events will inevitably give rise to loss of heterozygosity in somatic cells at an early age, resulting in a dominant pattern of disease transmission. Other autosomal dominant diseases act by dosage effects or dominant-negative actions, thus precipitating deleterious clinical sequelae. Hereditary triplet-repeat disorders—such as Huntington's disease, fragile X syndrome, and myotonic dystrophy—are caused by the expansion of a repeated 3-base sequence, which can occur in the regulatory or coding region of the affected gene, that is amplified in the germline from one generation to the next. This amplification fosters an increasingly severe phenotype in each successive generation (so-called **anticipation**). Finally, mitochondrial DNA disorders—such as Leber's optic neuropathy—are passed on maternally.

The common thread in these genetic diseases is the loss of function of a critical cellular component or the accumulation of a toxic gene product (see Table 49-2). Because these diseases are hereditary, the molecular and cellular defect is both widespread and lifelong. Traditional drug therapy is generally ineffective against this panel of disorders. Thus, these diseases represent an important opportunity for gene therapy strategies, which seek to replenish or counteract the defective function.

**TABLE 49-2** Examples of Diseases for Which Gene Therapy Has Been Tested or Is Being Considered

| CLASS | EXAMPLES OF DISEASE | MUTATED GENE PRODUCT | RESULT |
|---|---|---|---|
| Mendelian, homozygous recessive loss-of-function | Cystic fibrosis | CFTR, a cell-surface chloride channel | Deficient luminal water transport, salty sweat, pulmonary infections, male sterility, exocrine pancreas insufficiency, intestinal obstruction |
| | Familial hypercholesterolemia | Low-density lipoprotein (LDL) receptor | Extremely elevated plasma LDL, cholesterol, and triglycerides; xanthomas, xanthelasma; angina, early-onset acute myocardial infarction |
| | Tay-Sachs disease | Hexosaminidase A | Accretion of ganglioside GM2 in CNS neurons, apathy, retardation, blindness, convulsions, paralysis, macular degeneration, death by age 3 |
| Protein aggregation | Alzheimer's disease | *Tau* protein, amyloid precursor protein (APP) | Anterograde and retrograde amnesia; damage to cholinergic neurons in frontal and temporal lobes (especially hippocampus and amygdala) |
| Neoplastic | Malignant melanoma | B-Raf, cyclin dependent kinases and cdk inhibitors | Development of dysplastic cutaneous nevi, neoplastic transformation, eventual metastasis; target of "cancer vaccine" |
| Cardiovascular | NIDDM-induced small vessel disease | Polygenic disorder | Diabetic vasculitides, secondary to chronically elevated blood glucose, leading to paresthesias and neuritides; retinal angiogenesis leads to retinopathy and blindness |

## Protein Aggregation Diseases

The converse of the heritable loss-of-function diseases includes dominantly inherited disorders in which there is generation of an altered form of a particular gene product that has a dominant negative effect on normal function or a toxic gain of function. In the latter case, for example, the abnormal protein may accumulate in tissues, with toxic effects. A classic case is Alzheimer's disease. Though the precise etiology is still controversial, autopsies of Alzheimer's brains show both intracellular neurofibrillary tangles, or NFTs, of the microtubule-associated *tau* protein, and extracellular deposits of beta-amyloid protein (BAP). These gene products, as well as auxiliary proteins (e.g., apolipoprotein E4 and the presenilins), are often altered in Alzheimer's disease patients, and their dysregulation is believed to contribute to NFT formation and amyloid deposition. A similar pattern is observed in other amyloid accumulation disorders (e.g., amyloid kidney disease, diabetes mellitus type II, and systemic amyloidosis), and analogous processes are suspected in other adult-onset neurodegenerative illnesses such as amyotrophic lateral sclerosis (ALS). (In ALS, a somatically mutated superoxide dismutase I [SOD-I] gene may induce the pathology.) By reducing or abrogating production of the abnormal protein, gene therapy strategies such as antisense RNA or gene conversion could contribute enormously to the management of these disorders.

## Neoplastic Diseases

Cancer develops when a clonal population of somatic or germ cells possesses a combination of genetic and chromosomal aberrations that cause inappropriate cell cycling, inefficient DNA repair, loss of contact inhibition and apoptosis, and karyotypic instability. Because cancers originate in gain-of-function (oncogenic) and loss-of-function (tumor-suppressor) mutations in the genome, this class of diseases provides another potential target for gene therapy. Putative treatment strategies could focus on downregulating oncogenes or augmenting tumor-suppressor genes, or on selectively killing tumor cells directly or through enhanced T-cell mediated cytolytic attack. Some modalities unite gene therapy with more traditional small molecule-based approaches. For example, a vector expressing an enzyme that converts a prodrug into a toxic molecule could be transduced into tissues in the vicinity of a tumor, with the result that the neoplastic cells are selectively killed. Other strategies attempt to deliver apoptotic factors selectively to cancer cells, to differentiate the neoplastic cells, or to thwart the angiogenesis that is essential for continued growth or metastasis of a tumor. (Still another strategy, utilizing engineered adenoviruses as "oncolytic viruses," is discussed below.)

## Cardiovascular Diseases

Diseases of the heart and vascular system may be especially amenable to gene therapy strategies. Many disorders, such as the increasingly prevalent noninsulin-dependent (type II) diabetes mellitus (NIDDM), affect the vascular system in general, and loss of small-blood vessel capacity in particular, by inflammation, chemical complexation, and immune complex-mediated damage to vessel walls. This impairment of the microcirculation is the proximate cause of the peripheral neuropathies, renal disease, susceptibility to infection, and retinopathy of NIDDM. Sclerosed coronary arteries contribute to the ischemia of angina pectoris, caused by insufficient perfusion of the myocardium. After myocardial infarction, moreover, many patients suffer the complications of arrhythmias, congestive heart failure, and other chronic sequelae, in part because the infarcted region renders perfusion of the remaining cardiac muscle inadequate to maintain cardiac output. Although all of these disorders could benefit significantly from targeted revascularization, the efficacy of coronary angioplasty and coronary artery bypass surgery has been substantially hampered by restenosis in the former and vessel intimal proliferation in the latter. Some gene therapy strategies aim to promote revascularization and to suppress the complications of coronary artery manipulation, mainly by intervening in the signaling pathways that influence restenosis and intimal proliferation. Other modalities seek to stimulate angiogenesis directly at sites of cardiac or vascular injury, revascularizing or promoting the outgrowth of collateral circulation by delivering angiogenic factors locally. In practice, neovascularization produced in this fashion often lacks structural integrity and stability, limiting its clinical utility at the present time.

## Infectious Diseases

The most common application of gene therapy in the realm of infectious diseases is in the production of vaccines. Inoculation against microbial infection can be achieved by delivery of naked DNA that expresses epitopes specific for the pathogen. Viral vectors have also found utility. The same methods used to engineer viral vectors to express a missing gene can be applied to engender an immune response, by generating pathogen-specific proteins that stimulate humoral and cellular arms of the immune system. Some viruses (such as HIV) against which vaccination is desirable may be too dangerous to be administered as attenuated particles in healthy people, and strategies are, therefore, sought to express immunogenic epitopes in the context of other viruses. One possible frontier for gene therapy strategies may lie in confronting the increasing peril of microbial resistance to antibiotics and antiviral drugs. Persistent microbial evolution, high replication rates, and the paucity of pharmacologic targets in viruses, fungi, and some bacteria have combined to generate a formidable medical challenge for the coming century. Rapidly adaptable gene therapy modalities could contribute to more effective infectious disease management in the face of such challenges.

# REQUIREMENTS FOR SAFE AND EFFECTIVE GENE THERAPY

In the classic case of gene replacement, the objective of gene therapy is to modulate expression of an abnormal target gene, often by restoring the function of a mutated protein but sometimes by downregulating or controlling the expression of such a protein. Other applications (such as cancer therapy or angiogenesis) involve delivery of a novel gene to kill target cells selectively, or production of a factor to effect a change in the physiologic state of a damaged tissue. These modalities must meet several demands to be safe and effective.

1. Transgene persistence and long-term stable expression:
   (a) Expression of the transfected gene (supplied by the gene therapy vector in targeted cells) should be both steady and long term, with the transgene escaping degradation and epigenetic silencing.
   (b) Cells expressing the transduced gene should be protected from immune clearance.
   (It should be noted that the above two conditions apply primarily to the classical modality of gene replacement. In some applications, such as neovascularization, it may be desirable to curtail gene expression deliberately over time, whereas in cancer therapy, a vigorous immune response may be required for a succesful treatment strategy.)
2. Specific expression:
   Vectors should be directed to the target cell population of interest, and transduction of bystander tissues should be minimized.
3. Regulated expression:
   If the dosage of a restored normal gene must be precisely controlled (e.g., a hemoglobin gene in thalassemia), the gene therapy system should include a mechanism to manipulate expression levels reliably so as to approximate the physiologic state.
4. Reliable delivery:
   Parenterally administered, in vivo vectors should be sufficiently stable to reach the nucleus of target cells intact, evading plasma proteases and nucleases as well as lysosomal degradation and interferon-mediated suppression.
5. Minimal toxicity:
   Gene therapy vectors should have minimal toxicity, so as to minimize both immunogenicity and direct toxicity from the vector's components.

There are nearly 100 trillion cells in an adult human body, and directing a bolus of the nucleic acid "drug" to its targets is a formidable challenge. To circumvent obstacles to delivery, some gene therapy efforts that address heritable genetic diseases have focused on ex vivo transduction of hematopoietic and tissue stem cells, which are then reimplanted and utilized to restore the normal protein in the affected patient. In vivo gene therapy, in which the nucleic acid is administered parenterally, much like many small molecule drugs, requires the vector to home to the targeted tissues via the blood and lymphatic circulation or to be injected directly into the tissue. For both ex vivo and in vivo approaches, proper tissue targeting is essential for success. Moreover, because the immune system is designed to react to foreign proteins, such as those supplied in the gene therapy vector, delivery strategies must be designed either to evade immune mechanisms or to achieve effective delivery over a minimum number of treatment sessions before immune memory sets in. Finally, the pharmacodynamics and pharmacokinetics of in vivo gene therapy vectors are typically more intricate than those of small molecule drugs. Along with the traditional concerns of route of administration, plasma stability, and hepatorenal clearance, vectors must confront *immune* and *intracellular* clearance, and one must consider the body's handling of not only the DNA or RNA "drug" itself, but also the vehicle that ferries the drug into cells.

Because of their genomic modularity and innate ability to deliver genetic material into cells, viruses have become the vectors of choice for gene therapy. Recombinant DNA technology allows fragments of viral genomes to be maintained on separate DNA plasmids, permitting safe and convenient manipulation that supplants viral expression cassettes with the therapeutic genes of interest. The next section summarizes the salient features of viral vectors that have found application in gene therapy.

## VIRAL GENE THERAPY VECTORS: APPLICATIONS AND LIMITATIONS

Only a small subset of the immense variety of viruses have been tested as carriers of therapeutic genes. These have been chosen primarily from among the common human pathogens with broad tissue tropism, for which detailed information on genes and sequences is available. Viruses vary considerably in size, life cycle, titer, ease of laboratory manipulation, safety, immunogenicity, and composition (and they may possess RNA or DNA genomes). At the present time, nearly all gene therapy protocols use **retroviruses, adenoviruses, adeno-associated viruses (AAVs), herpesviruses,** or **Epstein–Barr virus (EBV)** as the gene delivery agent. The general features, advantages, and disadvantages of each virus family are summarized in Table 49-3.

### Retroviruses

Retroviruses (family *Retroviridae*) were so named because of their "retrograde" flow of genetic information from RNA in the virion to DNA in the host cells. Although many other RNA viruses exist, retroviruses stand out in their use of a DNA intermediate and in their possession of an RNA-dependent DNA polymerase called **reverse transcriptase** (RT). The notion of such an enzyme, an initially heretical idea, was first proposed by Howard Temin in the 1960s, and jointly discovered by Temin and David Baltimore in the 1970s. Crystallized in the early 1990s, reverse transcriptase is a $Mg^{2+}$ (or $Ca^{2+}$)-dependent multisubunit enzyme. RT is associated with RNase H, which degrades the template single-stranded (ss) RNA as it generates the double-stranded (ds) DNA intermediate. This DNA intermediate, demarcated by specialized regulatory repeats called the **long terminal repeats** (LTRs) on each end, then integrates randomly into the target cell's chromosomes. Integration is effected by a transposition reaction catalyzed by the viral **integrase** (IN). The virally coded DNA thus becomes an **integrated provirus.** A schematic depiction of retroviral integration into cellular DNA is shown in Figure 49-1. Although the provirus inserts itself nonspecifically (i.e., virtually anywhere in the genome), the ability of viral DNA to insert stably into the host genome has made retroviruses the workhorses of gene therapy.

In the case of a wild-type retrovirus, the provirus generates viral mRNAs that are translated into structural proteins (the *gag* polyprotein, which includes the matrix [MA], core shell [CA], RNA-associated [NC], and various other proteins of the nucleocapsid), enzymes (the *pro* [protease] and *pol* [RT and IN] gene products), and viral envelope proteins (*env*). The *pro-pol* region resides immediately downstream from the *gag*, and both gene products are produced from the same transcript. For the most part, translation terminates at the 3′ end of *gag*, but in roughly 1 out of 20 cases a read-through

TABLE 49-3 Viral Gene Therapy Vectors

| VIRAL VECTOR | TAXONOMY AND STRUCTURE | ADVANTAGES | DISADVANTAGES |
|---|---|---|---|
| Retrovirus | Retroviridae<br>ssRNA viruses<br>Reverse transcribe viral genome into dsDNA after entry into cells<br>~7–10 kb genome<br>Flanked by LTRs, which mark boundary between transgenic sequence and host genome | Reverse-transcribed DNA is integrated into host cell genome, providing the potential for long-term expression off retroviral U3 promoter or other viral or cellular promoters<br>Highly modular, can express *gag, pol, env* genes off separate plasmids and modify as desired<br>Replication-incompetent viral particles can be produced in packaging cells<br>No recombination steps required in construction | Transgene integration is random, creating a danger of interfering with crucial gene expression or activating protooncogenes (recently observed in clinical trial)<br>Some difficulties with long-term expression<br>Can infect only actively dividing cells after breakdown of the nuclear envelope (except lentiviruses such as HIV, which can infect resting cells) |
| Adenovirus | Adenoviridae<br>Linear dsDNA<br>30–40 kb genome<br>Sequential gene expression<br>Can infect many tissues<br>A cause of the common cold | High titers and efficiency of infection<br>E1a, E1b, E3, E4 regions can all be substituted with therapeutic genes; multiple inserts are therefore possible<br>High tropism for liver (useful for hepatic diseases) and various epithelia<br>Vectors can be complemented and packaged conveniently in cultured cells<br>Episomal residence and gene expression can be a safety feature | Construction requires viral recombination steps due to large size<br>Residual viral gene products (and transgene, to a lesser degree) elicit strong immune response against cells infected with the vector<br>Substantial difficulty achieving long-term expression off adenoviral episome, caused by immune clearance<br>Potential toxicity in infected cells, especially in liver<br>Danger of contamination with replication-competent helper virus in preparations |
| Adeno-Associated Virus (AAV) | Parvoviridae<br>Linear ssDNA<br>4.5–5.5 kb genome<br>Flanked by ITRs | Can transduce nondividing cells<br>Good transduction of CNS, muscle<br>Low immunogenicity<br>Availability of distinct serotypes that can infect different primary cell types | Small capacity limits size of packaged insert<br>Difficulty achieving large-scale production |
| Herpesvirus | Herpesviridae<br>Linear dsDNA<br>Very large (150 kb) genome; latency | Large size enables packaging of sizable inserts; e.g., potentially useful for treatment of DMD (an enormous gene)<br>Tropic for neural tissue | Some cellular damage observed even in replication-defective mutants<br>Silencing of promoters in CNS |
| Epstein–Barr Virus (EBV) | A herpesvirus<br>Circular DNA episome | Persistence of episome makes it useful in hybrid vectors | Low infection titers by itself, so not useful as a stand-alone gene therapy vector |

or antitermination event occurs at the *gag* stop codon, leading to production of the *gag-pro-pol* polyprotein. The *env* protein is generated by an alternative splicing event; complex retroviruses utilize such splicing to generate other proteins as well. The polyproteins are cleaved into their individual member proteins by the viral protease after a progeny virion has budded from an infected cell. Some virus strains also include regulatory genes. The lentiviruses, of which HIV is a member, retain a particularly baroque system of regulation in which genes such as *tat, rev, vif, nef,* and *vpu* influence mRNA elongation, splicing, nuclear-cytoplasmic transport, receptor degradation, and other functions.

In gene therapy vectors, the retroviral genes that are normally sandwiched between the LTRs are removed and re-

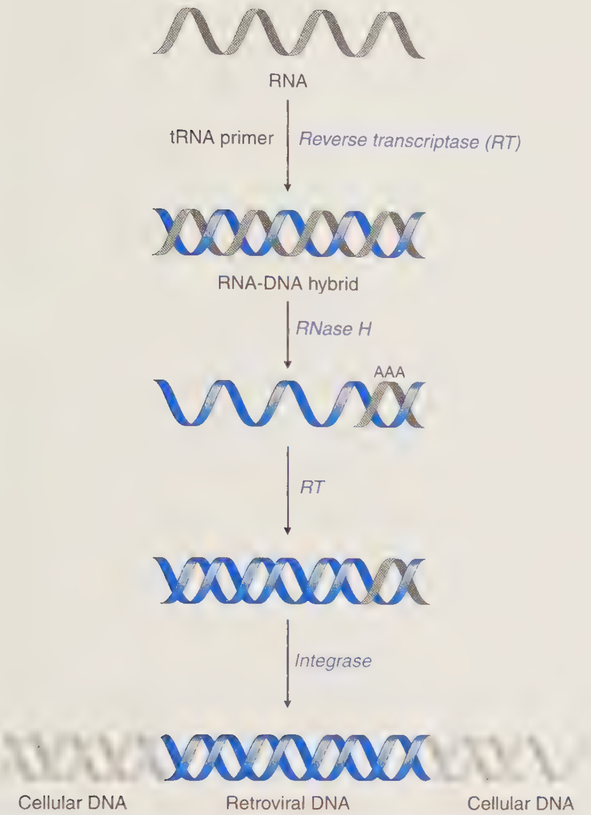


**Figure 49-1. Retroviral Integration into Cellular DNA.** A retroviral particle possesses two copies of an RNA genome, capped at the 5′-end and polyadenylated at the 3′-end (not shown). A tRNA primer carried in the viral particle binds to the RNA. Reverse transcriptase (RT) uses this tRNA primer to synthesize an RNA–DNA hybrid. RNase H degrades the RNA, and RT uses the remaining single-stranded DNA as a template to synthesize double-stranded viral DNA (blue). The viral-encoded integrase then catalyzes the insertion of the viral genome into cellular DNA (gray).

placed by the therapeutic gene of interest (Fig. 49-2). The LTR-containing vector, thus, resides on a DNA plasmid that can be readily modified for different applications. The U3 region of the LTR contains promoter and enhancer elements, and upon integration into a chromosome of the target cell, the therapeutic provirus will express its transgene off the 5′-LTR. This expression results in synthesis of the gene product of interest (e.g., a functional CFTR) in targeted cells. Expression off the U3 promoter is sometimes deliberately abrogated by self-inactivation, in which mutations introduced into the U3 of the downstream (3′) LTR are copied into the 5′ position during reverse transcription, abolishing U3 function in the provirus and generating so-called SIN (*s*elf-*in*activating) vectors. This allows transgene expression to be governed by a promoter internal to the 5′-LTR, often a different viral promoter [such as the strong cytomegalovirus (CMV) promoter] or a cellular one [usually for a ubiquitous housekeeping gene, such as chicken beta-actin (CBA) or phosphoglycerate kinase (PGK)]. Alternatively, such promoters can be introduced into the U3 of the 3′-LTR and copied directly into the 5′-position during reverse transcription. A number of other synthetic enhancements are frequently introduced into the proviral vector to augment nuclear-cytoplasmic transport or optimize nuclear localization to coordinate gene expression, such as the woodchuck hepatitis post-transcriptional regulatory element (WPRE) and scaffold/matrix attachment regions (S/MARs). Importantly, *removal of the normal retroviral genes renders the vector replication-incompetent;* that is, after transfecting a host cell, the vector is incapable of replicating itself. As detailed below, the sources of the complementing viral components are separated and maintained in distinct constructs and cell lines, so-called "helpers." In this way, the exclusion of viral genes from the LTR-containing plasmid provides a crucial safety margin when the retrovirus integrates into target cells; the resulting provirus expresses the therapeutic gene but cannot initiate replication.

Because the gene therapy vector itself is replication-incompetent, alternative strategies must be employed to manufacture the infectious virus. Additional plasmids are used to supply the *env* and *gag-pol* functions, generally expressing these proteins off strong nonretroviral promoters, such as the cytomegalovirus (CMV) or simian-virus 40 (SV40) promoters. These additional plasmids are often cotransfected with the therapeutic gene vector into a cell line, such as the SV40 T antigen-transformed human embryonic kidney line 293T. Such tripartite transfections, thus, provide the essential viral components in *trans* and the ensuing particles, which lack the *gag-pol,* and *env* sequences between their LTRs, cannot give rise to replicating viruses. Alternatively, an LTR-containing vector can be stably transfected into a **packaging** cell line that has been engineered to express the other critical components—the *gag-pol* and a viral envelope protein, such as the vesicular stomatitis virus envelope protein, that conveniently enables entry into a wide variety of cells. The resulting **producer** cell line will possess all the necessary viral components integrated into separate loci, thus functioning as a retroviral production factory when it is cultured. (Historically, such packaging and producer cell systems predated the practice of tripartite transfection.) The *env* and *gag-pol* mRNAs are then translated in the producer or packaging cell line, and the LTR-flanked therapeutic gene vector is packaged into retroviruses that are, crucially, nonreplicating. Recombination between the LTR-bearing vector and the complementing constructs is a potential hazard. The foregoing scheme for retroviral vector manufacture is diagrammed in Figure 49-2.

Interestingly, the use of retroviruses for gene therapy mimics an eons-old natural process in which infecting retroviruses have deposited nonreplicating versions of their genomes into human chromosomes, generating **human endogenous retrovirus sequences** (HERVs) that constitute 7%–8% of the human genome (a larger share than our own unique eukaryotic genes). Therapeutic genes delivered using a retroviral vector have the advantage of a stable, long-term presence in the recipient's somatic cells. In some cases, however, retroviral gene therapy has been hampered by transgene silencing over time. The precise mechanisms are still controversial, but there may be epigenetic modifications that downregulate expression off the novel locus. The expression at an integrated vector is subject to chromosomal influences, presumably because of the adjacent chromosomal transcriptional signals. Moreover, retroviruses in general can infect only cells that are actively cycling. Although this property is useful in targeting cancer cells, it poses a difficulty for other applications in vivo. Lentiviruses are able to infect nondividing as well as dividing cells, but the complexity and the potential toxicity of these viruses have thus far

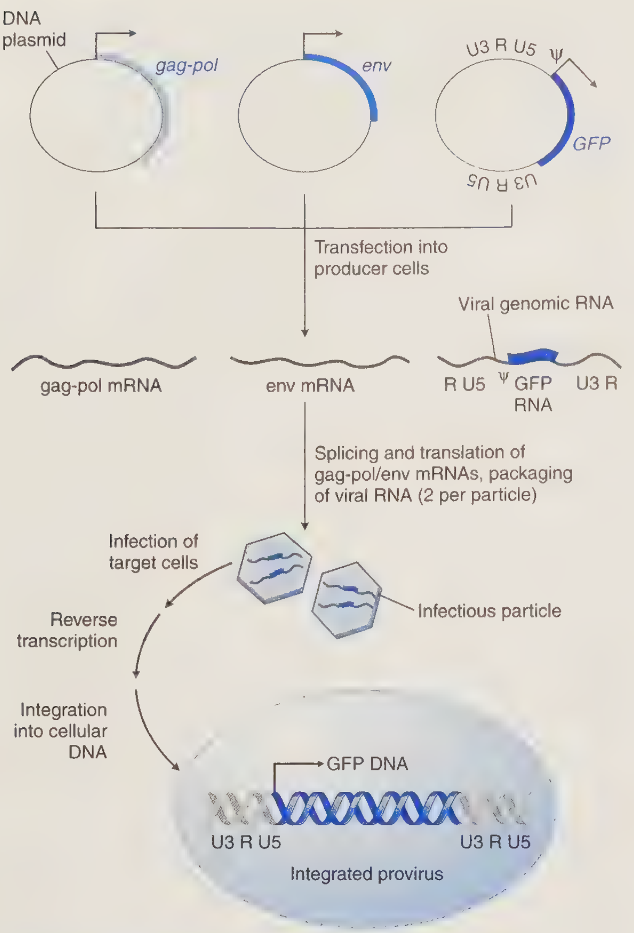

DNA plasmid
gag-pol
env
U3 R U5
Ψ
GFP
U3 R U5
Transfection into producer cells
Viral genomic RNA
gag-pol mRNA
env mRNA
R U5
Ψ GFP RNA
U3 R
Splicing and translation of gag-pol/env mRNAs, packaging of viral RNA (2 per particle)
Infection of target cells
Infectious particle
Reverse transcription
Integration into cellular DNA
GFP DNA
U3 R U5
U3 R U5
Integrated provirus

**Figure 49-2. Production of Recombinant DNA Using a Replication-Incompetent Retroviral Vector.** To replicate effectively, a virus requires structural proteins encoded by genes such as gag-pol and env. Only a sequence flanked by long terminal repeats (LTRs) is packaged in viral genomic RNA. Here, the LTR is represented by the sequence U3 R U5, and a gene encoding green fluorescent protein (GFP) is placed between two LTRs. Transfection of plasmids encoding gag-pol, env, and GFP results in production of gag-pol mRNA, env mRNA, and viral genomic mRNA containing the mRNA for GFP. Splicing and translation of the gag-pol and env mRNAs produces the proteins necessary to package the viral genomic RNA into infectious viral particles. These virions then infect target cells, where the genomic RNA undergoes reverse transcription and integration into cellular DNA (see Fig. 49-1). The integrated retrovirus—termed the proviral transgene—will, in this case, produce green fluorescent protein (GFP), a common laboratory reporter. For therapeutic applications, the GFP sequence could be replaced by that of a therapeutic gene.

restricted their use as human gene therapy vectors. Structural compaction of the nucleocapsid limits the amount of DNA that can be packaged by retroviruses—only 4–8 kb of extra sequence can be incorporated, making retroviruses ill-suited for cases (such as the dystrophin gene that is mutated in Duchenne muscular dystrophy) where a very large cDNA (14 kb) must be delivered.

In a recent clinical trial, Alain Fischer and colleagues in Paris used a retrovirus called Moloney murine leukemia virus to transduce dividing hematopoietic progenitors of patients with a rare X-linked immune disorder called SCID-X1. In this disorder, there is a deficiency in the γ-c subunit of the interleukin receptor that is critical for lymphocyte maturation. A functional γ-c gene was shuttled into the progenitors using the retroviral vector, and the patients' lymphocyte profiles were restored. Unfortunately, this ex vivo procedure, which is diagrammed in Figure 49-3, has very recently resulted in the emergence of a leukemia-like illness in several of the treated patients, apparently because of the activation of proto-oncogenes by adjacent retroviral integration, and the trial has been suspended.

Retroviruses have also been utilized as immunotherapeutic "**cancer vaccines.**" Melanoma cells are isolated from an affected patient and transduced with a retrovirus that confers expression of GM-CSF, a cytokine that spurs the activation of dendritic cells, the body's master antigen-presenting cells (APCs). The transduced melanoma cells are then reimplanted into the patient, inducing a tumor-specific immune response against nontransduced as well as transduced melanoma cells.

## Adenoviruses

Members of the family *Adenoviridae* are large (30–40 kb), linear dsDNA viruses that are frequently implicated in human

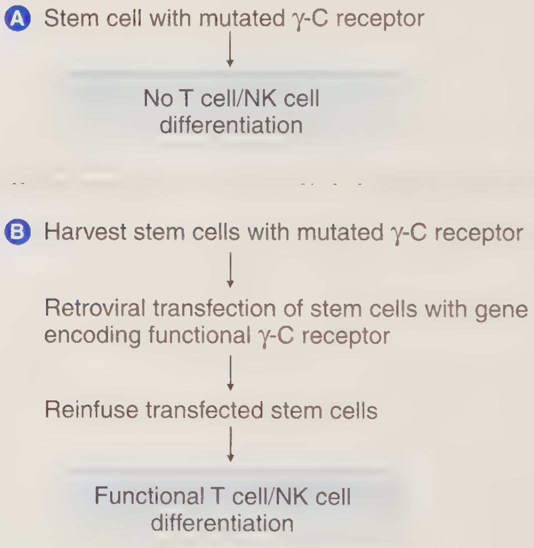

A Stem cell with mutated γ-C receptor
No T cell/NK cell differentiation
B Harvest stem cells with mutated γ-C receptor
Retroviral transfection of stem cells with gene encoding functional γ-C receptor
Reinfuse transfected stem cells
Functional T cell/NK cell differentiation

**Figure 49-3. Stem Cell-Based Gene Therapy.** **A.** Patients with severe combined immunodeficiency (SCID) possess a mutated γ-C receptor, resulting in a lack of T cell and NK cell differentiation. **B.** Stem cells harvested from a patient with SCID can be transfected retrovirally with a gene encoding a functional γ-C receptor. Insertion in the host genome is random. The transfected cells are then reinfused into the patient. In the presence of a functional γ-C receptor, the patient's immune system is able to produce functional T cells and NK cells. Use of the patient's own stem cells helps to obviate immune rejection of the transplanted cells, although the new gene can insert into (and, thereby, disrupt) an essential part of the genome. It may be possible to use similar methodology for a wide array of diseases, such as Duchenne muscular dystrophy, by differentiating stem cells to replace mutant tissues. Silencing of the retroviral transgene and toxicity associated with ectopic expression of the transgene are concerns.

illness, causing eye and bladder disorders as well as the common cold. The family is quite diverse, and there is frequent recombination among different serotypes, but the general organization is the same from virus to virus. *Adenoviridae* display a complex temporal regulation of gene expression divided into early (E), delayed early (IX and Iva2), and late (L) genes. Roughly speaking, early genes encode proteins essential to proper temporal regulation, whereas late genes code for the structural units that package new viral particles. Unlike retroviral genes, adenoviral sequences do not integrate except by a rare recombination event or at high multiplicities of infection; instead, they reside in the nucleus of an infected cell extrachromosomally. For gene therapy, this property can be a benefit or a shortcoming depending on the application, because gene expression will be transient. Each adenoviral genome is flanked on either side by an **inverted terminal repeat** (ITR). An origin of replication is located in the ITR, so this structure is also required for replication. Adenoviral genes are transcribed via recruitment of cellular RNA polymerase II.

The viral **E1a** and **E1b** genes have played an interesting role in cancer biology. Both human **Rb,** which is complexed by E1a, and human **p53,** which is complexed by E1b, are cellular checkpoint proteins that act as "watchdogs" to prevent inappropriate cell division and to regulate apoptosis. Adenoviruses can replicate only when Rb and p53 are inactivated. Viruses in which E1a and E1b have been deleted can, therefore, be used to replicate selectively in and, thereby, lyse cells deficient in Rb and/or p53. Importantly, a large proportion of human cancers is deficient in one or both of these checkpoint proteins. The clinical use of such "oncolytic" adenoviruses has been stymied thus far by problems with efficient vector delivery to tumors, but this approach holds promise as a potentially selective treatment modality.

For several reasons, adenoviruses have been the vectors of choice in many gene therapy applications. Novel inserts can be introduced into E1, E2, and parts of the E4 region, with about 20 kb of sequence available for the incorporation of foreign DNA. (This is a larger amount than is available in retroviruses.) Adenoviruses can be produced conveniently in very high titers in the human 293 cell line, which has been transformed with E1a and E1b and thus complements a replication-incompetent adenoviral vector. Although adenoviruses have a partial tropism for particular tissues (especially the liver), their host range is broad and is applicable to a variety of targets. Finally, the ability of adenoviruses to transduce both dividing and nondividing cells is a key advantage for in vivo applications. One promising application of gene therapy exploits these features of adenoviral vectors to express immunostimulatory epitopes in vaccine systems.

On the downside, adenoviruses are notorious for stimulating a vigorous immune response against infected cells. This is an obstacle to both efficacy and safety, and the cytotoxic response to adenovirus-transduced cells has been a persistent obstacle to long-term transgene expression. (So-called **gutless** or **helper-dependent** adenoviral vectors, in which the vector is depleted of viral sequences, have been utilized to circumvent this issue and increase capacity to 36 kb.) There is also the ongoing challenge of ensuring the absence of replication-competent "helper" particles in gene therapy preparations.

## Adeno-Associated Viruses (AAVs), Herpesviruses, Epstein–Barr Virus

AAVs are a family of small, 4.5–5.5 kb single-stranded DNA parvoviruses flanked by ITRs. They derive their name from the fact that they require the adenoviral E1a, E1b, E2a, and E4 gene products *in trans* to propagate themselves, and therefore are naturally infectious only in tandem with a concurrent adenoviral infection of a target cell. In practice, because replication-deficiency is desirable in a gene therapy vector, adenoviral complementation is furnished only in the cells in which the AAV vectors are initially generated. AAVs have attracted particular interest as vectors for brain and muscle, but are being tested in a wide array of other organs as well. Multiple serotypes of the AAVs exist, which can provide a variety of options to confront a wide array of clinical challenges. Their potential tissue range, moreover, is enhanced by their capacity to infect nondividing cells. They can integrate into target DNA like retroviruses, and are even capable of doing so in a site-specific manner. The AAV *rep* protein recognizes a cognate sequence that results in enhanced integration into a region of the long arm of chromosome 19 called the AAVS1 site. In practice, however, AAV vectors do not express *rep* after infection of target cells, and, therefore, integrate randomly (like retroviruses), either as single proviruses or as head-to-tail concatemers. Interestingly, recent studies suggest that in vivo gene transfer of AAV vectors most frequently leads to stable expression of gene products from an unintegrated rather than an integrated state. Despite the advantages—low toxicity and the ability to infect resting cells—AAVs are limited by their small packaging size. Large-scale production has also been particularly challenging for these vectors.

Herpesviruses are enormous (>150 kb) dsDNA viruses, among the largest found in nature. They are ubiquitous in human disease: herpes simplex virus (HSV)-1 causes cold sores and fever blisters, HSV-2 induces genital pruritus and blisters, varicella-zoster virus (VZV) causes both chicken pox and shingles, and human herpesvirus (HHV)-8 induces AIDS-associated Kaposi's sarcoma. Herpesvirus recombinant vectors can accommodate up to 30 kb of inserted sequence, and so-called **amplicons** (which are analogous to gutless adenoviruses) can incorporate up to 150 kb of foreign DNA sequence. Thus, herpesviruses provide one of the only viable means to package very large gene cassettes, such as a cDNA for the dystrophin gene (which is mutated in Duchenne muscular dystrophy). Herpesviruses are also tropic for the nervous system, which they target by retrograde axonal flow from infection at a mucosal surface to reside in a dorsal root ganglion. This property makes herpesviruses valuable for treatment modalities that must surmount the blood–brain barrier. Furthermore, their propensity to enter a state of latency following infection helps to reduce immune provocation and facilitate long-term establishment in target cells. Like other vectors, herpesviruses suffer from the potential for gene silencing in transduced cells (including silencing of derived "latency" promoters). Moreover, use of even nonreplicating herpesviruses can have some toxicity.

One interesting herpesvirus variant is the Epstein–Barr virus (EBV), the pathogen that causes infectious mononucleosis. As a virus with transforming potential that is tropic for lymphocytes, EBV is also implicated in Burkitt's lymphoma and nasopharyngeal carcinoma. EBV is unusual in that it persists in infected cells as a circularized episome, and it partitions—along with the host cell genome—into dividing cells following replication. Its titers are generally low and, although it is impractical as a gene therapy vector by itself, EBV is popular as a component of so-called "hybrid vectors" that utilize two or more viral backbones to generate the vector. An adenoviral-EBV vector, for example, combines the high efficiency and wide host range of the adenoviruses with the episomal persistence of EBV, to forge a "virus within a virus." EBV elements are packaged within an adenoviral "shell," then excised following infection by a lox-CRE mediated system to liberate the episome. Adenovirus- or herpesvirus-retrovirus hybrids have a similar objective, introducing retroviral integration elements into the genome of the adenovirus or herpesvirus to form an integrating component.

## Antisense RNA and RNA Interference

A final application for gene therapy consists of a set of strategies that employ RNA molecules to inhibit a gene of interest selectively; for example, a gene whose product is toxic. Toxic proteins or peptides are thought to be involved in degenerative diseases of the nervous system, such as Huntington's disease, amyotrophic lateral sclerosis, Kreutzfeld–Jakob disease, retinitis pigmentosa, and (probably) Alzheimer's disease. In such cases, the protein or peptide accumulates (often by aggregation) at levels that are toxic to nearby neurons, and therapeutic approaches directed at minimizing the deleterious accumulation may be beneficial. Cancer is another target of RNA inhibitory approaches because neoplasia inevitably involves the inappropriate and constitutive expression of growth-enhancing and cell-cycling genes whose repression could potentially have therapeutic value. In addition, RNA inhibitory strategies against cancer may be more adaptable than other modalities in treating relapses caused by new mutations in tumor cells, because an RNA molecule that targets its novel (mutated) complement is far easier to generate than a small molecule or peptide that targets a protein. Finally, RNA inhibition could potentially yield a rich harvest of new antimicrobial drugs that are targeted against the wealth of unique gene products present in foreign pathogens.

From a physiologic perspective, "RNA inhibition" consists of a series of conserved mechanisms, in both prokaryotes and eukaryotes, that regulate gene expression and combat viral infection by modulating mRNA amounts and controlling the levels of mRNA translation. RNA inhibition entails two overlapping yet distinct modalities: (1) **antisense RNA,** and (2) **RNA interference** or **RNAi.** (The action of ribozymes, in which catalytic RNA cleaves an RNA target in *cis* or in *trans,* is also related to these modalities.) There is occasional confusion between the two, in part because of their similarities, but antisense RNA is not the same as RNAi and, in fact, the latter was discovered as a consequence of research on the former.

The principle of antisense RNA is elementary, relying on the ability of RNA, like DNA, to form duplexes. Thus, an RNA transcript elaborated from the antiparallel strand of an open reading frame in the genome will hybridize to the mRNA produced upon activation of transcription at that gene. Watson–Crick pairing of a capped and polyadenylated message with its complement can then interfere with mRNA processing, ribosome interactions, and/or the docking of translation initiation factors, effectively rendering the mRNA incompetent for translation. Interaction of a transcript with its antisense sequence can also promote RNAse H mediated degradation of the message (Fig. 49-4). The principle of antisense inhibition was established in the early 1980s, and it found utility as a laboratory tool for selective blocking of gene expression in a wide variety of cell types and organisms. Physiologically, antisense RNAs were shown to play important roles in the control of transcription and genome replication in prokaryotes. The role of antisense transcripts in eukaryotic cells is more nebulous; to date, such mechanisms have been linked to modulation of N-myc and thymidylate synthase levels and to the control of eukaryotic translation initiation factors and developmental proteins. Interestingly, eukaryotic antisense transcripts have also been implicated in the phenomenon of genomic imprinting, which involves the selective, epigenetic downregulation of alleles specific to the paternal or maternal chromosome. Antisense RNA approaches have recently been applied in the

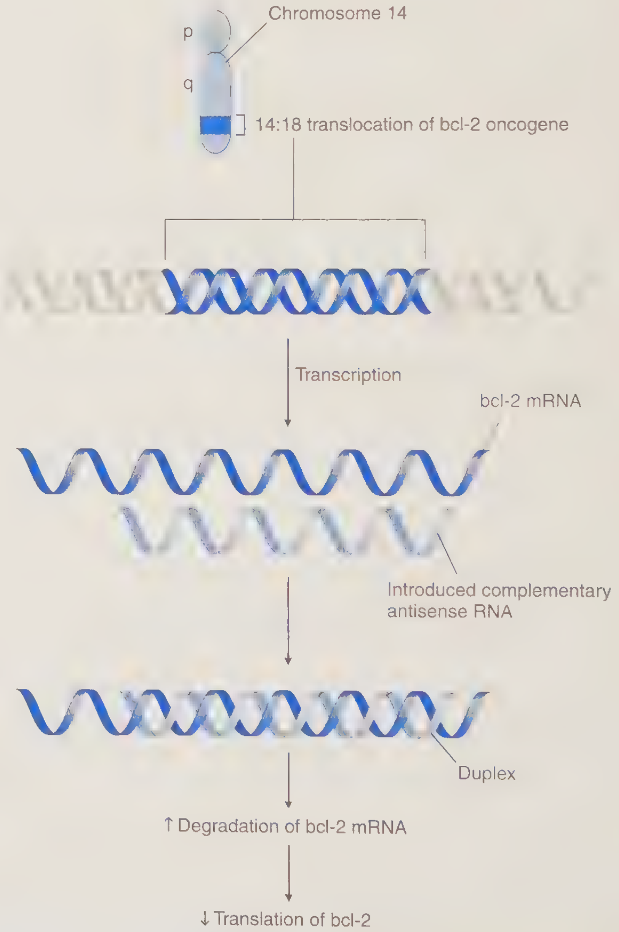

Chromosome 14
p
q
14:18 translocation of bcl-2 oncogene
Transcription
bcl-2 mRNA
Introduced complementary antisense RNA
Duplex
↑ Degradation of bcl-2 mRNA
↓ Translation of bcl-2

**Figure 49-4. Use of Antisense RNA Molecules to Block Production of an Oncogenic Gene Product.** A characteristic 14:18 translocation results in production of the bcl-2 oncogene. The translocated region of DNA is shown in blue. This region is transcribed into bcl-2 mRNA. Administration of complementary antisense RNA (light blue) results in formation of an RNA–RNA duplex. The RNA duplex is preferentially degraded by the cell. The decreased level of bcl-2 mRNA results in decreased translation of bcl-2.

clinic; **fomivirsen** is a recently approved antisense RNA used to treat retinitis caused by cytomegalovirus in patients immunocompromised by HIV infection. The design of therapeutic antisense RNA is complex, and its use in vivo is often associated with nonspecific immune stimulation.

Like antisense RNA, RNAi requires the formation of a dsRNA structure. Otherwise, however, the two mechanisms are remarkably different. In plants, the discovery of RNAi stemmed from the observation that petunias supplied with an exogenous copy of the chalcone synthetase gene did not, as expected, boost the gene's activity uniformly, but instead suppressed it. In *C. elegans* embryos, Andrew Fire and colleagues reported in 1998 that a mixture of sense and antisense RNA strands was particularly effective at selective inhibition of gene expression. In *Drosophila,* a dsRNA substrate several hundred base pairs in length can be processed by a dsRNA-specific endonuclease, called Dicer, into short fragments approximately 20 bp long. These short dsRNAs, called siRNAs (for *s*mall *i*nterfering RNA particles), are the active species in RNAi. The siRNAs form a triplex RNA structure with homologous mRNAs, presumably through transient melting or "breathing" of the duplex, which allows Watson–Crick base-pairing between the siRNA and the target. The siRNA–mRNA complex draws the attention of the RISC (*R*NA-*i*nduced *s*ilencing *c*omplex) array, which digests the target mRNA. In this way, mRNAs containing sequences identical or homologous to the siRNAs are selectively degraded. RNAi has been shown to be operational across many species, including plants, *C. elegans, Drosophila,* and mammals.

RNA inhibition strategies represent a potentially exciting frontier because of their power and selectivity in downregulating target genes. One drawback, of course, is that they act primarily at the level of elaborated RNA transcripts rather than the genome itself. Thus, continuous administration would be required to inhibit a targeted gene chronically. RNA inhibition also represses gene expression incompletely, and as with all gene therapy strategies, successful delivery is a serious challenge. Nonetheless, the RNAi approach embodies many of the facets that make gene therapy potentially valuable—in particular, the ability to modify the drug rapidly and adapt it to changes (such as mutations) in disease targets. RNAi has already demonstrated promise in cell culture, thwarting the infection of cells by HIV and polio virus. Moreover, the Dicer-directed endonuclease processing step can be bypassed to enable direct synthesis and utilization of the active siRNA, and methodologies to express siRNA species directly off pol III or phage promoters may permit continuous expression of these inhibitory molecules inside cells of interest through viral vectors.

## Conclusion and Future Directions

Modern medicine stands at the edge of an entirely new pharmacology. For the first time in history, physicians seek to manage disease at the level of the genetic and protein information that underlies all biology.

Protein-based therapies are playing an increasingly important role in the pharmacologic treatment of disease. The potential for new therapies is virtually endless, given the thousands of proteins produced by the human body and the many thousands of proteins produced by other organisms. The early success of recombinant insulin production in the 1970s created an atmosphere of enthusiasm and hope, which was, unfortunately, followed by an era of disappointment when the vaccine attempts, nonhumanized monoclonal antibodies, and cancer trials in the 1980s were largely unsuccessful. Despite these setbacks, significant progress has recently been made. The large number of protein therapies both in current clinical use and in phase III clinical trials attests to the promise of this technology. It is likely that novel protein-based therapies will be available shortly for many types of cancer, autoimmune disorders, neurological diseases, graft rejection, microbial diseases, and vascular and hematologic regulation. In fact, recombinant human proteins now make up the majority of FDA-approved biotechnology medicines, which include monoclonal antibodies, natural interferons, vaccines, hormones, modified natural enzymes, and various cell therapies. Exciting new means of production, such as genetically engineered plants, are changing the scale, cost, and even route of administration of recombinant protein therapies. For example, plants can inexpensively produce vast quantities of protein without waste or bioreactors, and potatoes can be engineered to express recombinant proteins and thereby to make edible vaccines. One can safely predict that there will be an expanding role for protein therapies in the future, although the success of any particular protein therapy will depend on its pharmacodynamic, toxic, and pharmacokinetic profiles.

The promise of gene therapy resides in its potentially high specificity and large therapeutic index, as well as in the potential for rapid adaptation of therapies on an individualized basis. The latter property may be especially valuable in confronting cancer and a wide array of infectious diseases, for, as has become evident over the past several decades, natural selection is as operative in microbial and cellular pathology as it is in animal biology. The success of new chemotherapeutic drugs is too often attenuated or overcome altogether by the rise of resistant clones; the long-term solution to this challenge may be the capacity of the pharmacologist and clinician to respond just as rapidly. At the present time, gene therapy faces giant hurdles in terms of vector delivery, stable transgene expression, and safety. But as research to overcome these obstacles progresses, the potential for both ex vivo and in vivo gene therapy comes closer to realization and clinical application.

## Suggested Readings

### Protein-Based Therapy

Banting FG, Best CH, Collip JB, et al. Pancreatic extracts in the treatment of diabetes mellitus. Can Med Assoc J 1922;12:141–146. (*Describes the treatment of diabetes by an extract from pancreas—later shown to be insulin.*)

Clark M. Antibody humanization: a case of the "Emperor's new clothes"? Immunol Today 2000;21:397–402. (*Details the different methods of engineering chimeric antibodies.*)

Finn OJ. Cancer vaccines: between the idea and the reality. Nat Rev Immunol 2003;3:630–641. (*Review of cancer vaccine production with examples of recombinant protein use.*)

Glennie MJ, Johnson PW. Clinical trials of antibody therapy. Immunol Today 2000;21:403–410. (*Reviews the therapeutic antibodies in use and under development.*)

Grabowski GA, Barton NW, Pastores G, et al. Enzyme therapy in type 1 Gaucher disease: comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. Ann Intern Med 1995;122:33–39. (*Trial reporting the efficacy of recombinant glucocerebrocidase in the treatment of Gaucher's disease.*)

Liotta LA, Espina V, Mehta AI, et al. Protein microarrays: meeting analytical challenges for clinical applications. Cancer Cell 2003; 3:317–325. (*Describes the use of large numbers of proteins simultaneously to diagnose clinical conditions.*)

Russell CS, Clarke LA. Recombinant proteins for genetic disease. Clin Genet 1999;55:389–394. (*Discusses the role of proteins in treating genetic disease.*)

### Gene Therapy

Brantl S. Antisense-RNA regulation and RNA interference. Biochim Biophys Acta 2002;1575:15–25. (*Summary of the principal mechanisms and applications of RNA inhibition.*)

Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000;288:669–672. (*Landmark paper describing a successful strategy to treat a congenital immunodeficiency disease with retroviral gene therapy. Unexpected complications in treated patients have since prompted changes to the initial approach.*)

Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA. 1993;90: 3539–3543. (*Application of retroviral gene therapy tools to prime cytotoxic T cells against neoplastic targets, so-called "tumor vaccination."*)

Hannon GJ. RNA interference. Nature 2002;418:244–251. (*Review of the RNAi phenomenon: its history, salient characteristics, and applicability as a research tool.*)

Jacobs A, Breakefield XO, Fraefel C. HSV-1-based vectors for gene therapy of neurological diseases and brain tumors: part II. Vector systems and applications. Neoplasia 1999;1:402–416. (*Summary of herpesvirus-based vector systems as tools in the treatment of central nervous system diseases.*)

Lam PY, Breakefield XO. Hybrid vector designs to control the delivery, fate and expression of transgenes. J Gene Med 2000; 2:395–408. (*Review of hybrid-vector schemes, utilizing sequences from multiple viral sources, in gene therapy, detailing both the benefits and drawbacks of several approaches thus far devised.*)

Mulligan RC. The basic science of gene therapy. Science 1993;260: 926–932. (*Review of the scientific bases and challenges of clinical gene therapy. The issues raised by this review from a decade ago remain relevant today.*)

**TABLE 49-1** (expanded) A Functional Classification of Protein-Based Therapies

| CATEGORY (r, RECOMBINANT; p, PURIFIED) | PROTEIN | TRADE NAME | FUNCTION | EXAMPLES OF CLINICAL USE |
|---|---|---|---|---|
| **Group I. Protein Therapeutics with Enzymatic or Regulatory Activity** | | | | |
| **Ia. Replaces a Protein That Is Deficient or Abnormal** | | | | |
| rIa | Factor VIII | Bioclate, Helixate, Kogenate, Recombinate, ReFacto | Coagulation factor | Hemophilia A |
| rIa | Factor IX | Benefix | Coagulation factor | Hemophilia B |
| rIa, rIb | Insulin | Humulin, Novolin | Regulates blood glucose, shifts potassium into cells | Diabetes mellitus, diabetic ketoacidosis, hyperkalemia |
| rIa, rIb | Growth hormone (GH) | Genotropin, Humatrope, NorIVitropin, Nutropin, Protropin, Siazen, Serostim | Anabolic and anticatabolic effector | Growth failure caused by GH deficiency or chronic renal insufficiency, Prader–Willi syndrome, Turner syndrome, AIDS wasting, or cachexia with antiviral therapy |
| rIa | β-Glucocerebrosidase | Cerezyme | Hydrolyzes glucocerebroside to glucose and ceramide, recombinant | Gaucher's disease |
| pIa | β-Glucocerebrosidase | Ceredase | Hydrolyzes glucocerebroside to glucose and ceramide, purified from pooled human placenta | Gaucher's disease |
| pIa | Pancreatic enzymes (lipase, amylase, protease) | Arco-Lase, Cotazym, Pancrease, Viokase, Creon, Zymase, Donnazyme | Digests food (protein, fat, and carbohydrate), purified from hogs and pigs | Poor digestion, gas, bloating, pancreatic insufficiency, steatorrhea, cystic fibrosis, chronic pancreatitis, post-Billroth II gastric bypass surgery, pancreatic duct obstruction |
| pIa | Lactase | Lactaid | Digests lactose, purified from fungus *Aspergillus oryzae* | Gas, bloating, cramps, diarrhea due to inability to digest lactose |
| pIa | Adenosine deaminase | Adagen<br>Pegademase bovine, PEG-ADA | Metabolizes adenosine, prevents accumulation of adenosine, purified from cows | Severe combined immunodeficiency disease (SCID) caused by adenosine deaminase (ADA) deficiency |
| pIa | α1-Proteinase inhibitor | Prolastin | Inhibits elastase-mediated destruction of pulmonary tissue, purifed from pooled human plasma | Congenital α1-antitrypsin deficiency |

*(continued)*

**TABLE 49-1** (expanded) A Functional Classification of Protein-Based Therapies *(Continued)*

| CATEGORY (r, RECOMBINANT; p, PURIFIED) | PROTEIN | TRADE NAME | FUNCTION | EXAMPLES OF CLINICAL USE |
|---|---|---|---|---|
| **Group Ib. Augments an Existing Pathway** | | | | |
| Fertility | | | | |
| rIb | Human chorionic gonadotropin (HCG) | Ovidrel | Stimulates ovarian follicle rupture and ovulation | Assisted reproductive technology for infertility |
| rIb | Human follicle-stimulating hormone (FSH) | Gonal-F / Follistim | Augments ovulation | Assisted reproductive technology for infertility |
| Hematopoiesis | | | | |
| rIb | Erythropoietin | Epogen, Procrit | Stimulates erythropoiesis | Anemia of chronic disease, myleodysplasia, anemia caused by renal failure or chemotherapy, preoperative preparation |
| rIb | Granulocyte-macrophage colony stimulating factor (GM-CSF) | Leukine, Sargramostim | Stimulates proliferation and differentiation of neutrophils, eosinophils and monocytes | Leukopenia, myeloid reconstitution post bone marrow transplantation, HIV/AIDS |
| rIb | Granulocyte colony stimulating factor (G-CSF) | Filgrastim | Stimulates neutrophil proliferation, differentiation and migration | Neutropenia in AIDS or post chemotherapy or bone marrow transplantation, severe chronic neutropenia |
| rIb | Interleukin-11 (IL-11) | Neumega<br>Oprelvekin | Stimulates megakaryocytopoiesis and thrombopoiesis | Prevention of severe thrombocytopenia, especially after myelosuppressive chemotherapy |
| Immunoregulation | | | | |
| rIb | Type I alpha-interferon | Infergen | Unknown, immunoregulator | Chronic Hepatitis C virus infection |
| rIb | Interferon alpha-2a (α2a) | Roferon-A | Unknown, immunoregulator | Hairy cell leukemia, chronic myelogenous leukemia |
| rIb | Interferon alpha-2b (IFN α2b) | Intron A<br>Peg-Intron | Unknown, immunoregulator<br>Recombinant interferon alpha-2b with polyethylene glycol (PEG) added to increase half-life | Condylomata acuminata, chronic hepatitis B, chronic non-A, non-B hepatitis, hairy cell leukemia, melanoma, Kaposi's sarcoma<br>Chronic hepatitis C virus infection in patients 18 years or older with compensated liver disease who have not been treated previously with IFN-alpha |
| rIb | Interferon beta-1a (rIFN-β) | Avonex<br>Betaseron | Unknown, antiviral, and immunoregulator | Multiple sclerosis |

| | | | | |
|---|---|---|---|---|
| rIb | IFN-γ | Actimmune | Increases inflammatory and antimicrobial response | Chronic granulomatous disease (CGD), severe osteopetrosis |
| rIb | Interleukin-2 (IL-2), epidermal thymocyte activating factor (ETAF), aldesleukin | Proleukin | Stimulates T and B cells, natural killer cells, and lymphokine-activated killer (LAK) cells | Metastatic renal cell cancer, melanoma |
| Hemostasis and Thrombosis | | | | |
| rIb | Factor VIIa | Novo-Seven | Coagulation factor | Hemophilia A or B with bleeding |
| rIb | Alteplase (tissue plasminogen activator (tPA)) | Activase | Promotes fibrinolysis by binding fibrin and converting plasminogen to plasmin | Pulmonary embolism, acute myocardial infarction, acute ischemic stroke |
| rIb | Reteplase (deletion mutein of plasminogen activator (rPA)) | Retavase | Contains the nonglycosylated kringle 2 and protease domains of human tPA, functions similarly to tPA | Management of acute myocardial infarction, improvement of ventricular function |
| rIb | Dotrecogin alfa (activated protein C) | Xigris | Anti-thrombotic (inhibits coagulation factors Va and VIIIa), anti-inflammatory | Severe sepsis with a high risk of death |
| Other | | | | |
| rIb | Teriparatide (human parathyroid hormone 1–34) | Forteo | Administered as a once-daily injection, markedly enhances bone formation | Severe osteoporosis |
| rIb | Platelet-derived growth factor (PDGF) | Regranex | Promotes wound healing by enhancing granulation tissue formation and fibroblast proliferation and differentiation | Debridement adjunct for diabetic ulcers |
| rIb, rIV | Glucagon | GlucaGen | Pancreatic hormone that increases blood glucose by stimulating the liver to convert glycogen to glucose | Hypoglycemia, diagnostic aid to slow gastrointestinal motility in radiographic studies |
| pIb | L-asparaginase | ELSPAR, ONCOSPAR | Provides exogenous asparaginase activity, removing available asparagine from serum, purified from *E. coli* | Acute lymphocytic leukemia (ALL), which requires exogenous asparagine for proliferation |

*(continued)*

**TABLE 49-1** (expanded) A Functional Classification of Protein-Based Therapies *(Continued)*

| CATEGORY (r, RECOMBINANT; p, PURIFIED) | PROTEIN | TRADE NAME | FUNCTION | EXAMPLES OF CLINICAL USE |
|---|---|---|---|---|
| pIb | Trypsin | Granulex | Proteolysis | Decubitus ulcer, varicose ulcer, debridement of eschar, dehiscent wound |
| rIb | Nesiritide | Natrecor | Recombinant B type natriuretic peptide | Acute decompensated congestive heart failure |
| **Group Ic. Provides a Novel Function or Activity** | | | | |
| rIc, rIa | Darbepoietin alfa | Aranesp | Modified erythropoietin with longer half life, stimulates red blood cell production | Treatment of anemia in patients with chronic renal insufficiency and chronic renal failure (± dialysis) |
| rIc, rIa | Tenecteplase | TNKase | Tissue plasminogen activator with greater specificity for plasminogen conversion, has amino acid substitutions of Thr103 to Asp, Asp117 to Gln, and Ala for amino acids 296–299, promotes fibrinolysis by binding fibrin and converting plasminogen to plasmin | Acute myocardial infarction |
| rIc | Etanercept | Enbrel | Dimeric fusion protein between recombinant soluble tumor necrosis factor receptor (TNFr) and Fc portion of human IgG1 | Moderate to severe active rheumatoid arthritis (RA) after failing other therapies, moderate to severe active polyarticular juvenile RA |
| rIc | Hirudin | Lepirudin | Thrombin inhibitor from salivary gland of medicinal leech *Hirudo medicinalis* | Heparin induced thrombocytopenia (HIT) syndrome |
| rIc | Dornase alfa | Pulmozyme | Human deoxyribonuclease | Cystic fibrosis, decreases respiratory tract infections in selected patients with FVC greater than 40% of predicted |
| pIc | Streptokinase | Streptase | Converts plasminogen to plasmin, degrades plasmin inhibitors, produced by group C β-hemolytic streptococci | Acute evolving transmural myocardial infarction, pulmonary embolism, deep vein thrombosis, arterial thrombosis or embolism, occlusion of arteriovenous cannula |

| | | | | |
|---|---|---|---|---|
| pIc | Papain | Accuzyme, Panafil | Protease from the *Carica papaya* fruit | Debridement of necrotic tissue or liquefication of slough in acute and chronic lesions, such as pressure ulcers, varicose and diabetic ulcers, burns, postoperative wounds, pilonidal cyst wounds, and carbuncles |
| pIc | Collagenase | Collagenase Santyl | Collegenase obtained from fermentation by *Clostridium histolyticum,* digests collagen in necrotic base of wounds | Debridement of chronic dermal ulcers and severely burned areas |
| **Group II. Protein Therapeutics with Special Targeting Activity**<br>**IIa. Interferes With a Molecule or Organism** | | | | |
| rIIa | Rituximab | Rituxan | Chimeric (human/mouse) mAb that binds CD20, a transmembrane protein found on over 90% of B-cell non-Hodgkin's lymphomas | Treatment of relapsed or refractory, low-grade or follicular, CD20-positive B-cell non-Hodgkin's lymphoma (NHL) |
| rIIa | Gemtuzumab ozogamicin | Mylotarg | Humanized anti-CD33 IgG4 kappa monoclonal antibody conjugated to calicheamicin (a chemotherapeutic agent) | Relapsed CD33-positive acute myeloid leukemia in patients who are more than 60 years old and are not candidates for cytotoxic chemotherapy |
| rIIa | Alemtuzumab | Campath | Humanized monoclonal antibody against CD52 antigen on T and B cells | B-cell chronic lymphocytic leukemia (B-CLL) in patients who have been treated with alkylating agents and who have failed fludabarine therapy |
| rIIa | Infliximab | Remicade | Monoclonal antibody that binds and neutralizes TNF-α, preventing induction of proinflammatory cytokines, changes in endothelial cell permeability, activation of eosinophils and neutrophils, induction of acute phase reactants, and enzyme elaboration by synoviocytes and/or chondrocytes | Rheumatoid arthritis, Crohn's disease |

*(continued)*

UNIVERSITY COLLEGE CORK Library

**TABLE 49-1** (expanded) A Functional Classification of Protein Based Therapies *(Continued)*

| CATEGORY (r, RECOMBINANT; p, PURIFIED) | PROTEIN | TRADE NAME | FUNCTION | EXAMPLES OF CLINICAL USE |
|---|---|---|---|---|
| rIIa | Muromonab-CD3 | Orthoclone/OKT3 | Monoclonal antibody that binds CD3 and blocks T cell function | Treatment of patients with acute renal allograft rejection or steroid resistant cardiac or hepatic allograft rejection |
| rIIa | Daclizumab | Zenapax | Humanized IgG1 mAb that blocks cellular immune response in graft rejection by binding the alpha chain of CD25 (IL-2 receptor) and thereby inhibiting the IL-2 mediated activation of lymphocytes | Prophylaxis against acute allograft rejection in patients receiving renal transplants |
| rIIa | Basiliximab | Simulect | Chimeric (human/mouse) IgG1 that blocks cellular immune response in graft rejection by binding the alpha chain of CD25 (IL-2 receptor) and thereby inhibiting the IL-2 mediated activation of lymphocytes | Prophylaxis against allograft rejection in renal transplant patients receiving an immunosuppressive regimen including cyclosporine and corticosteroids |
| rIIa | Abciximab | ReoPro | Fab fragment of chimeric (human/mouse) mAb 7E3 that inhibits platelet aggregation by binding to the glycoprotein IIb/IIIa integrin receptor | Adjunct to aspirin and heparin for prevention of cardiac ischemia in patients undergoing percutaneous coronary intervention or patients about to undergo percutaneous coronary intervention with unstable angina not responding to medical therapy |
| rIIa | Palivizumab | Synagis | Humanized IgG1 mAb that binds the A antigenic site of the F protein of the respiratory syncytial virus | Prevention of respiratory syncytial virus infection in high risk pediatric patients |
| pIIa | Ovine digoxin immune sera, Fab portion | Digifab | Monovalent fragment antigen binding (Fab) immunoglobulin fragment obtained from sheep immunized with a digoxin derivative | Treatment of cardiac patients with digoxin toxicity |
| **Group IIb. Stimulates a Signaling Pathway** | | | | |
| rIIb | Anakinra | Kineret, Antril, Synergen | Recombinant interleukin-1 receptor antagonist | Moderate to severe active rheumatoid arthritis in adults who have failed one or more disease modifying anti-rheumatic drugs |

| | | | | |
|---|---|---|---|---|
| rIIb, rIIa | Trastuzumab | Herceptin | Monoclonal antibody that binds Her2/Neu cell surface receptor | Breast cancer |
| **Group IIc. Delivers Other Compounds or Proteins** | | | | |
| Currently in Clinical Trials | | | | |
| **Group III. Protein Vaccines** | | | | |
| **IIIa. Protects Against a Deleterious Foreign Agent** | | | | |
| rIIIa | OspA | LYMErix | Noninfectious lipoprotein on outer surface of *Borrelia burgdorferi* | Lyme disease |
| rIIIa | HBsAg | Engerix, Recombivax HB | Noninfectious protein from surface of Hepatitis B virus | Hepatitis B |
| **IIIb. Treats an Autoimmune Disease** | | | | |
| rIIIb | Glatiramer acetate (formerly copolymer-1, consists of acetate with L-Glu, L-Ala, L-Tyr, L-Lys) | Copaxone | Unknown, modifies immune response, may not act by classical vaccine mechanisms | Relapsing-remitting multiple sclerosis |
| **IIIc. Treats Cancer** | | | | |
| Currently in Clinicai Trials | | | | |
| **Group IV. Protein Diagnostics** | | | | |
| rIV | DPPD | Recombinant purified protein derivative (DPPD) | Noninfectious protein from *Mycobacterium tuberculosis* | Diagnosis of tuberculosis exposure |
| rIV | HIV antigens | Enzyme immunoassay (EIA), western blot | Detects human antibodies to HIV | Diagnosis of HIV infection |
| rIV | Hepatitis C antigens | Recombinant immunoblot assay (RIBA) | Detects human antibodies to hepatitis C virus | Diagnosis of hepatitis C exposure |
| rIV, rIa | Growth hormone-releasing hormone (GHRH) | Geref | Stimulates growth hormone (GH) release by somatotroph cells of the pituitary gland | Diagnosis of defective growth hormone secretion |
| rIV | Thyroid stimulating hormone (TSH) | Thyrogen | Stimulates thyroid epithelial cells or well-differentiated thyroid cancer tissue to take up iodine and produce and secrete thyroglobulin, triiodothyronine, and thyroxine | Adjunctive diagnostic for serum thyroglobulin testing in the follow-up of patients with well-differentiated thyroid cancer |

# 50

# Drug Delivery Mechanisms

Joshua D. Moss and Robert Langer

**Introduction**
**Case**
**Use of Novel Delivery Routes**
  Oral Delivery
  Pulmonary Delivery
  Transdermal Delivery
**Polymer-Based Delivery Systems**
  General Mechanisms
    *Diffusion*
    *Chemical Reaction*
    *Solvent Activation*
  Intelligent Delivery
  Targeting
**Liposome-Based Delivery Systems**
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Drugs are typically administered in either pill or injection form, with limited control over release-rate and localization. More advanced drug delivery systems have recently been developed, however. The goal of these new technologies is to alter four pharmacokinetic properties: (1) absorption of the drug, including the period of time over which it is released into the systemic circulation or at its final site of action; (2) distribution of the drug, whether it be to the entire body or to a specific tissue or organ system; (3) metabolism of the drug, either to be avoided entirely or used to convert the drug to an active form; and (4) elimination of the drug.

This chapter describes several existing and emerging delivery technologies, and discusses how these technologies influence one or more of these four properties. The field of drug delivery is large and encompasses many disciplines, and this discussion will highlight approaches that illustrate these properties, rather than provide an exhaustive description of all ongoing practice and research. The techniques described include novel use of already successful delivery routes, polymer-based delivery, and liposome-based delivery.

## ■ Case

*March 1988:* Mr. F. is 13 years old. His parents begin to notice that he is tired much of the time, despite getting plenty of sleep. He can no longer participate on his school's track team because he becomes exhausted in the middle of races—the same races he had often won less than a year earlier. Also, Mr. F. complains of being thirsty constantly and, as a result, consumes large quantities of water. Mr. F. goes to his family physician, who measures his blood glucose level at 650 mg/dL (approximately six times normal levels) and makes an initial diagnosis of type I diabetes mellitus. The diagnosis is confirmed in the hospital, where Mr. F. stays for 1 week while physicians stabilize his blood glucose and develop an insulin therapy regimen. He is taught how to draw a drop of blood from his fingertip to measure his blood glucose and how to give himself subcutaneous injections of insulin. Each day, Mr. F. injects recombinant human insulin before breakfast and before dinner.

*January 1997:* Throughout high school and most of college, Mr. F. rarely monitors his glucose levels and purposely keeps them higher than recommended. He wants to be as "normal" as possible, which for him means never allowing his glucose level to fall so low as to require food in the middle of a class or at other unusual times. As Mr. F. gets older, he begins to appreciate that avoiding the long-term consequences of poorly controlled diabetes—atherosclerosis, retinopathy, nephropathy, and peripheral neuropathy, among others—is worth the inconvenience of better control. He switches to a four injection per day regimen and begins checking his blood glucose four to five times per day. Eventually, he switches from multiple subcutaneous injections (MSI) to continuous subcutaneous insulin infusion (CSII) with an insulin pump. The pump delivers a constant basal level of insulin that can be supplemented with bolus injections before meals, thereby more closely approximating the body's normal control of blood glucose levels.

*September 2014:* Back in 1997, Mr. F. used his insulin pump for only about 3 months, deciding that the small machine he needed to keep constantly attached to his body was not compatible with his active lifestyle or self-image. He resumed MSI therapy for several more years, until he began participating in human trials for a new, implantable insulin delivery system. Now, a 2-year supply of insulin is incorporated into a polymer matrix that can be implanted in the subcutaneous fat of the abdomen. A device in Mr. F.'s wristwatch constantly measures his glucose levels transdermally, and it transmits instructions to a magnetic oscillator implanted near the polymer delivery system. The dosing advantages of the insulin pump are thus achieved without Mr. F. feeling limited or tied to a machine in any way. He simply has the polymer system replaced every 2 years and makes minor daily adjustments to the programmed delivery parameters in his wristwatch device. Mr. F. is looking forward to receiving a transplant of pancreatic beta cells, developed from his own stem cells, that will completely cure his diabetes.

## QUESTIONS

■ **1.** Why is oral administration of insulin not practical? What other routes could be tried?

■ **2.** How can polymers be used to optimize and simplify administration of some drugs?

## USE OF NOVEL DELIVERY ROUTES

### ORAL DELIVERY

Oral administration of small molecules is currently the most common method of drug delivery. The main advantages of oral delivery are ease of use and relatively low cost, both of which can improve patient compliance. However, incomplete absorption, metabolism of the drug during absorption, and metabolism of the drug during the first pass through the liver can adversely affect bioavailability of the drug. The variability of these factors, as well as limitations in dosing frequency, also affect the ability to maintain a therapeutic drug concentration in the blood. In addition, only relatively small molecules can be used in conventional pills: the intestine generally cannot absorb large molecules intact. Intact peptide and protein drugs, such as insulin, are poorly absorbed orally because of proteolysis in the digestive tract. Recent advances and ongoing research in oral drug delivery are beginning to address these issues.

Sustained or **extended release formulations** can improve the patient's ability to maintain a prolonged plasma drug concentration with less frequent doses. In early approaches to sustained release, pill or capsule solubility was modified with one or more inert substances known as **excipients.** By formulating the drug in an emulsion or suspension that is relatively difficult to digest, the period of time over which the drug dissolves and is absorbed can be extended. Similar results have been achieved by coating the drug with substances such as cellulose derivatives or wax. This approach is used in a wide variety of both prescription and over-the-counter medicines. Another successful and more recent approach to sustained release oral formulations involves an osmotic pump capsule (discussed below).

Techniques are also being researched for delivery of larger molecules, such as proteins and DNA, in oral formulations. Several designs make use of drug-carrying vehicles, including liposomes and microspheres. **Liposomes,** small vesicles with lipid bilayer membranes, are lipophilic and can be taken up by intestinal Peyer's patches when targeted to M-cells (specialized epithelial cells) with appropriate ligands. Certain types of liposomes have been moderately successful in experimental oral vaccine delivery; their use in intravenous delivery systems is discussed below. Polyanhydride **microspheres,** which adhere strongly to the intestinal mucosal surface, have been shown to penetrate intestinal epithelium. After the microspheres are absorbed, presumably because they can stay in contact with the intestinal epithelium for long periods of time, the complex molecules carried within them can be released into the blood.

Another potential approach to delivering proteins orally involves targeting the drug to the colon, which has lower levels of protease activity than upper portions of the gastrointestinal tract. For example, microsphere delivery vehicles can be synthesized from polymers that have enzymatically degradable azoaromatic crosslinks. The colon has a relatively high concentration of azoreductases, resulting in degradation of the microspheres and protein release within the colon. Substances that transiently increase the permeability of colonic epithelium, possibly coincorporated in the microspheres, may improve the absorption of proteins once delivered to the colon.

### PULMONARY DELIVERY

Patients suffering from asthma and other respiratory diseases have long been able to treat their condition by inhaling aerosols of drugs directly into their lungs; $\beta_2$-adrenergic agonists, such as albuterol, and glucocorticoid analogues are widely used examples of such drugs. In early metered-dose inhaler designs, many of which are still used, the drug is delivered in liquid form using a high velocity chlorofluorocarbon (CFC) propellant. With this technique, very little drug is reproducibly delivered to the lung–often less than 10%. Particles often accumulate in the mouth and throat, and many are immediately exhaled. Components of the immune system and macrophages in the lung can also clear some of the drug before it can act. In addition, many patients use their inhalers incorrectly; common mistakes include not shaking the inhaler well enough, pressing the inhaler too early or too late during inhalation, or using an empty inhaler. Incorrect use further reduces delivery efficiency.

Inhaler design continues to improve. Recent advances include more consistent dosing, greater ease of use via electronic breath actuation, and non-CFC propellants. The aerosol formulations have also been improved by adjusting several properties of the particles themselves. For example, optimized particle chemistry and surface morphology can minimize undesirable particle–particle aggregation. Similarly, particle solubility can be modified to influence the rate of therapeutic release once delivered. Dry-powder aerosol clouds that reach deep into the lungs can be generated by blowing compressed air into a drug powder, breaking the powder into tiny (1–5 μm) particles inside the inhaler.

The lung also offers several potential advantages for noninvasive, systemic delivery of molecules. The large alveolar surface area, thin tissue lining, and limited numbers of proteolytic enzymes make the lung an ideal location for proteins and peptides to enter the bloodstream. Many biotherapeutics that are currently administered subcutaneously—such as growth hormone, insulin, glucagon, and $\alpha_1$-antitrypsin—are being investigated for inhalation therapy. In addition, both dosing frequency and cost can be reduced for local applications of pulmonary delivery, such as treatment of asthma and cystic fibrosis.

One approach to achieving increased delivery efficiency is the design of large, highly porous aerosol particles with very low densities. Such particles tend to aggregate less than smaller, denser particles, resulting in more efficient aerosolization. In addition, these particles have an "aerodynamic diameter," a parameter based on both density and actual particle dimensions, similar to that of conventional aerosol particles; thus, they can reach the deep parts of the lung through an airstream despite their relatively large (5–20 μm) size. Once deposited, the particles can escape clearance by alveolar macrophages, because phagocytosis of particles by macrophages diminishes with increasing particle size beyond 2–3 μm. Thus, drugs can be delivered more efficiently over longer periods of time. In one study, insulin was encapsulated in biodegradable polymer microspheres. Some of the microspheres were small and nonporous, and some were large and porous (low density), but both types had similar aerodynamic diameters. Relative bioavailability of the large, porous insulin particle when delivered into the lungs was about seven times greater, and the total time of insulin release into the systemic circulation was about 24 times longer, than that of the conventional particle.

## TRANSDERMAL DELIVERY

The stratum corneum, composed of lipids and keratinocytes, comprises the outermost skin layer and the major barrier to transdermal transport. Small, lipophilic drugs have been successfully delivered through the skin into the systemic circulation by passive diffusion at low flux rates, thereby avoiding first-pass metabolism by the liver. Currently, passive transdermal patches are available for pharmacologic treatment of motion sickness, angina, nicotine withdrawal, hypertension, pain, for hormone replacement, among others. In addition to providing greater bioavailability while remaining noninvasive, transdermal delivery systems are often associated with fewer adverse side effects, such as liver damage during first-pass metabolism, than conventional oral dosage forms. Thus, transdermal systems that provide these advantages for drug molecules normally unable to penetrate the skin are under active development.

**Iontophoresis** is one approach to enhancing transport of charged low molecular mass molecules, such as pain medications, through the skin. It involves application of low-voltage electrical pulses for long time periods, and it is already used clinically for local applications, such as therapy for hyperhidrosis (excessive perspiration). In addition, the use of high-voltage pulses for a short time period—on the order of milliseconds—is being explored. In human cadaver skin, which is commonly used as a model for skin transport, these high-voltage pulses have been used to induce temporary pores. This phenomenon, known as **electroporation,** potentially allows delivery of large, charged molecules, such as heparin and oligonucleotides.

Ultrasound enhancement of drug delivery through the skin, termed **sonophoresis,** is also being explored for molecules such as insulin, interferon, and erythropoietin. Application of ultrasound to the skin results in cavitation, the formation of tiny air-filled spaces in lipid bilayers of the stratum corneum. The net result of cavitation is disordering of the lipid bilayers, enhancing diffusivity of the drug through the skin by up to 1,000 times. Sonophoresis does not damage the skin, which typically resumes normal structure within 2 hours, and no undesirable effects have been observed in early human tests.

Sonophoresis can also be used to remove diagnostic samples from the extracellular space through the skin. Experiments have been devised in which a reservoir was placed between an ultrasound transducer and a rat's skin, and interstitial fluid was extracted. Theophylline, glucose, cholesterol, urea, and calcium could be measured in the sample; the glucose measurements were accurate enough to be used as a surrogate for blood glucose monitoring in diabetics. With a portable ultrasound transducer, this technique could be incorporated in a futuristic device like the one Mr. F. used in 2014.

# POLYMER-BASED DELIVERY SYSTEMS

## GENERAL MECHANISMS

Polymer-based drug delivery systems gradually release drugs into their surroundings. Polymer delivery mechanisms are widely used in diverse applications such as birth control, chemotherapy, and antiarrhythmic therapy. These systems offer advantages in both controlled release and targeting of drugs, and are thus the focus of much research. Drug delivery from a polymer-based system can be achieved via three general mechanisms: (1) diffusion; (2) chemical reaction; and (3) solvent activation (Fig. 50-1).

### Diffusion

**Diffusion** from either a reservoir or a matrix is the most common release mechanism. In a reservoir system, the drug is

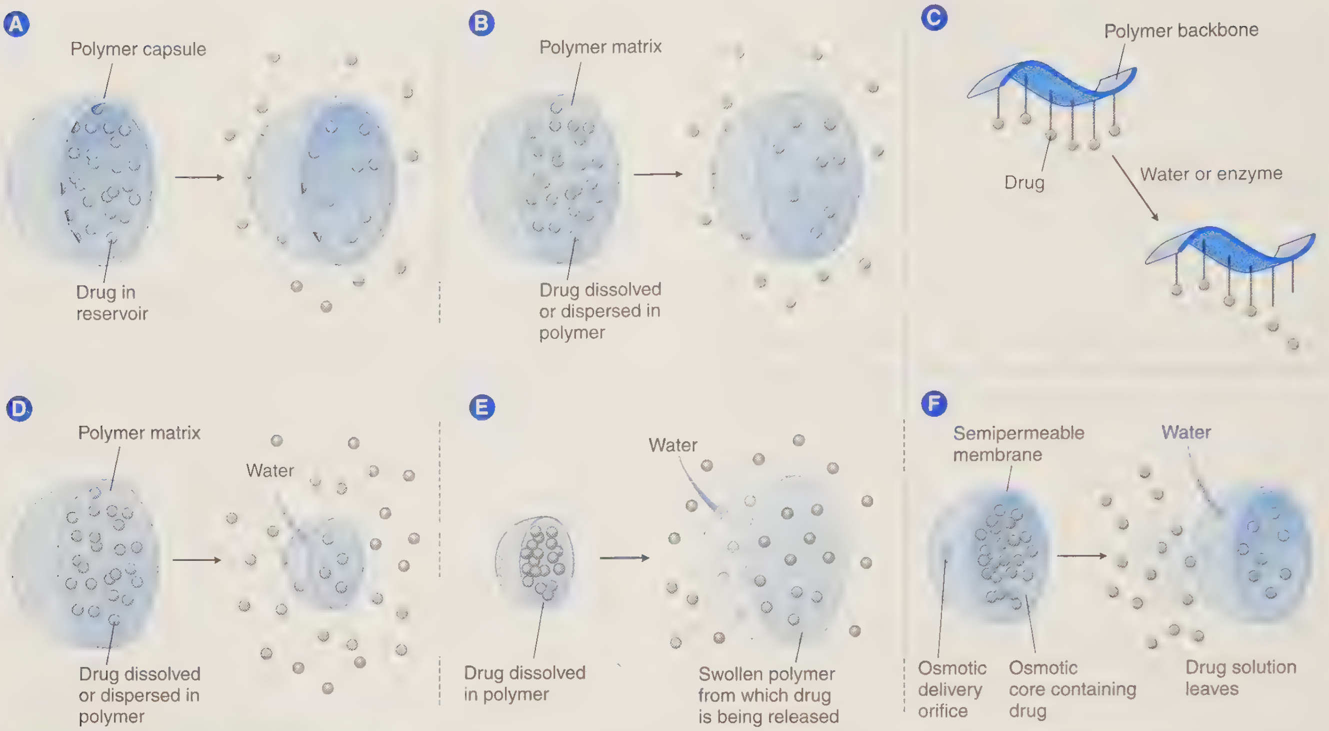


**Figure 50-1. Polymer Release Mechanisms.** In all panels except (**C**), the simplified diagrams represent polymeric systems in cross section. The most common release mechanism is diffusion, whereby the drug migrates from its initial location in the polymer system to the polymer's outer surface and then to the body. Diffusion can occur from a reservoir (**A**), in which a drug core is surrounded by a polymer film, or from a matrix (**B**), where the drug is uniformly distributed through the polymeric system. Drugs can also be released by chemical mechanisms such as cleavage of the drug from a polymer backbone (**C**) or hydrolytic degradation of the polymer (**D**). Exposure to a solvent can also activate drug release. For example, the drug can be retained in place by polymer chains; upon exposure to environmental fluid, the outer polymer regions begin to swell, allowing the drug to diffuse outward (**E**). An osmotic system in the form of a tablet with a laser-drilled hole in the polymer surface (**F**) can provide constant drug release rates. Water diffuses through the semipermeable membrane into the tablet along its osmotic gradient, swelling the osmotic core inside the tablet and forcing drug solution out through the hole. Combinations of the above approaches are possible. Release rates can be controlled by the nature of the polymeric material and the design of the system.

contained within a polymer membrane through which it diffuses over time (Fig. 50-1A). Norplant, a long-term contraceptive system, acts by this principle. **Levonorgestrel,** a synthetic progestin, is stored in small silicone tubes implanted in the arm. The drug diffuses slowly through the polymer capsule over the course of 5 years, providing effective long-term contraception (for a further discussion of progestin action on the menstrual cycle, see Chapter 27, Pharmacology of Reproduction). Reservoir systems are, however, limited by the size of the drug molecules being delivered. Molecules larger than approximately 300 Daltons are unable to diffuse through the polymer shell.

In one common matrix system design, the drug is contained in a series of interconnecting pores within the polymer, rather than in one large reservoir. This system is less limited by the size of the drug molecules because each pore can accommodate molecules with molecular weights of several million Daltons. The rate of diffusion between the pores—and thus through the matrix and out of the system—is controlled architecturally; tight constrictions and tortuous connections between pores prevent rapid release of the stored drug. One such system is used clinically to administer **gonadotropin releasing hormone (GnRH) analogues.** GnRH analogues are peptide hormones that, when administered continuously, inhibit anterior pituitary gland production of gonadotropins (LH and FSH) and are useful in the treatment of sex-hormone dependent diseases, such as prostate cancer. A major previous limitation of this therapeutic approach was the short in vivo half-life of GnRH analogues following intramuscular injection. When the drug is incorporated into polymer microcapsules, and the capsules are injected intramuscularly, the lifetime of GnRH is effectively extended, so that therapeutic concentrations are maintained over a period of from 1–4 months. In addition to the drug diffusing out of the microcapsules, the polymer matrix itself begins to degrade slowly once injected, further facilitating drug release. Thus, the microcapsule system uses a second mechanism of polymer-based drug delivery, chemical reaction, in this case between the polymer and water.

## Chemical Reaction

In **chemical reaction-based systems,** part of the system is designed to degrade over time, because of either a chemical or enzymatic reaction. In some designs, covalent bonds that connect the drug to a polymer are cleaved in the body by endogenous enzymes (Fig. 50-1C). Such polymer–drug complexes are typically administered intravenously, and the use of water-soluble polymers such as polyethylene glycol (PEG) increases biological half-life of the drug considerably. For example, PEG-Intron™, a pegylated form of **interferon-α2b,** has been approved by the FDA for weekly administration; this treatment for hepatitis C previously required injections three times as often. In the case of the intramuscular GnRH microcapsules discussed above, the polymer itself is degraded in a reaction with water (Fig. 50-1D).

Most insoluble polymers considered for these applications exhibit bulk erosion (i.e., the entire matrix dissolves at the same rate), which results in larger pores and a more sponge-like, less stable structure. This pattern of degradation makes constant release rates difficult to achieve and creates the potential risk of undesirable dose dumping. Novel polymers have been designed to overcome this problem by degrading optimally (i.e., through surface erosion) for controlled drug delivery. For example, a polymer with desirable erosion properties can be engineered by using hydrophobic monomers connected by anhydride bonds. The hydrophobic monomers exclude water from the interior of the polymer matrix, eliminating bulk erosion. The anhydride bonds, however, are highly water reactive, allowing surface erosion once implanted. This design allows the polymer to degrade from the outside only (Fig. 50-2). The actual rate of degradation can be controlled by using a combination of monomers, one more hydrophobic than the other. The length of time that the polymer persists is specified by the ratio of monomers used, and a drug that is uniformly distributed within such a polymer matrix will be released constantly over time. Based on these principles, Gliadel® has become the first controlled-release system for an anticancer drug to receive FDA approval. After surgeons remove recurrent glioblastoma multiforme, an aggressive form of brain cancer, they place up to eight small polymer-drug wafers at the tumor site. As the polymer surface erodes over 1 month, the drug **carmustine** (a DNA alkylating agent; see Chapter 32, Pharmacology of DNA Replication and Mitosis) is slowly released. The concentration of carmustine at the tumor site is maintained at a level high enough to kill the remaining tumor cells, while the harmful side effects of systemic delivery are avoided. This treatment significantly prolongs the lives of patients with this form of brain cancer.

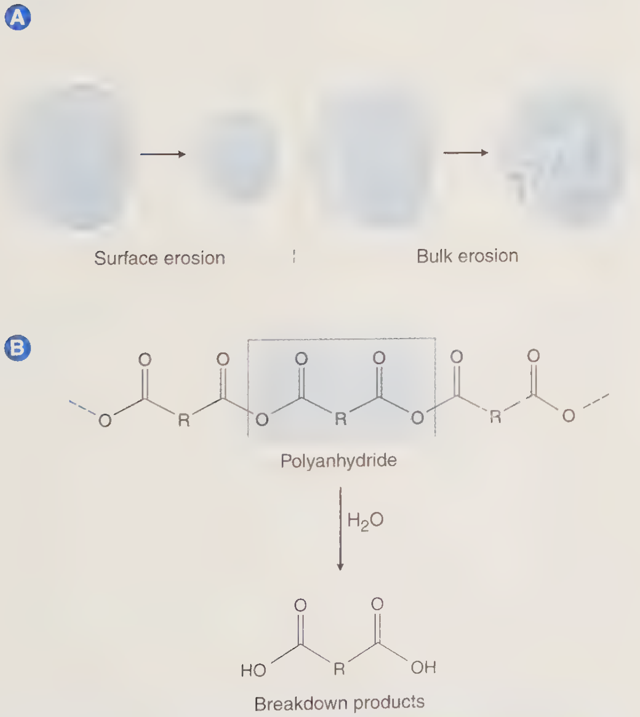


**Figure 50-2. Surface Erosion Using Polyanhydride Polymers.** **A.** Surface erosion of degradable polymer delivery devices allows for more accurately controlled release rates and is, therefore, preferable to bulk erosion. **B.** Polyanhydrides are used to promote surface erosion. They have hydrophobic monomers that exclude water from the interior of the polymer matrix and prevent bulk erosion. However, the monomers are linked by water-soluble anhydride bonds, allowing breakdown at exposed surfaces.

## Solvent Activation

The third mechanism for polymer-based drug delivery is **solvent activation,** in which the solvent does not react with the polymer chemically, but rather initiates drug release via **osmosis** (Fig. 50-1E) or swelling (Fig. 50-1F) of the system. One widely used example of such a system is the oral administration of **nifedipine,** a calcium channel blocker (see Chapter 20, Pharmacology of Vascular Tone). The drug is mixed with an osmotically active agent, such as a salt, and coated with a membrane that is permeable to water but not the drug. A small hole is then drilled in the capsule membrane with a laser. After ingestion, the constant osmotic influx of water through the membrane forces the drug out of the pill through the hole, thereby controlling release. This delivery technique, when compared to conventional oral formulations, provides patients greater relief from ischemic events with fewer adverse side effects. Concerta®, an extended release formulation of **methylphenidate,** uses a similar system to treat children with attention deficit hyperactivity disorder (ADHD).

# INTELLIGENT DELIVERY

There are situations in which pulsatile delivery is desirable to mimic the body's natural pattern of producing chemicals. In the case of Mr. F., the insulin pump he wore briefly provided a constant, basal rate of insulin to maintain his blood glucose levels between meals. When Mr. F. ate, he could set the pump to provide a bolus of insulin, therefore preventing a sudden, excessive rise in blood glucose concentration. Several innovative approaches have been taken to incorporate such versatility in polymer-based drug delivery systems, which have been more traditionally designed to deliver drugs at constant or decreasing release rates.

In one early design, magnetic beads were incorporated in the polymer matrix with a 2-year supply of insulin. The system was then implanted subcutaneously in rats, where the insulin was slowly released by diffusion out of the matrix, as discussed above. When an oscillating magnetic field was applied externally, movement of the magnetic beads within the matrix caused alternate expansion and contraction of the drug-carrying pores. The insulin could thus be effectively squeezed out of the matrix, resulting in higher dose delivery for as long as the oscillating magnetic field was applied. This system significantly lowered blood glucose levels in the rats compared to control rats, and may eventually become a viable method of insulin delivery. In Mr. F's hypothetical future, the magnetic oscillator was implanted; he could quickly administer a bolus of insulin simply by selecting the appropriate program on his wristwatch controller, which sent the instructions to the implanted device via a radiofrequency signal.

Other methods of increasing the rate of drug diffusion from a polymer matrix include application of either ultrasound or electric current. Ultrasound delivered at an appropriate frequency can have an effect similar to that of the magnetic bead system. Ultrasound causes cavitation—the formation of tiny pockets of air—in the polymer, disrupting the porous architecture to facilitate faster drug release. Applying an electric current to certain polymers can induce electrolysis of water at the polymer surface, lowering local pH and disrupting hydrogen bonding within the complex. The polymer subsequently degrades at a faster than normal rate, allowing transient release of larger drug doses. Pulsatile delivery can also be achieved via local environmental stimuli. For example, hydrogels—materials composed of polymers and water—can be designed to sense changes in temperature, pH, and even specific molecules by virtue of their structure.

A silicon microchip delivery system that offers even more control over release rates has recently been designed. The microchip contains up to 1,000 tiny drug reservoirs, each covered with a thin gold film. Applying a small voltage to an individual reservoir dissolves the gold film electrochemically, releasing the drug stored in that reservoir. Because the reservoirs can be loaded and opened individually, almost limitless possibilities exist for both dosing of single drugs and combining multiple drugs.

## TARGETING

Accurate targeting allows for larger, more effective doses to reach the tissues of interest without risking the toxic side effects of systemic delivery. When targeting tissues, the first variable controlled is the anatomic placement of the polymer-based drug delivery system; the carmustine wafer delivery system discussed above makes use of this basic consideration. Other notable examples include Estring, a vaginal ring that delivers **estradiol** for vaginal dryness; Vitrasert, an eye implant that delivers **ganciclovir** for the treatment of cytomegalovirus retinitis in AIDS patients (see Chapter 34, Pharmacology of Viral Replication); and drug-eluting stents that deliver **sirolimus** for the prevention of in-stent restenosis in coronary angioplasty (see Chapter 43, Pharmacology of Immunosuppression). Many tissues are accessed practically only via the bloodstream, however, making targeted delivery more difficult. Both passive and active targeting techniques have been developed to direct polymer-based systems that circulate in the bloodstream to specific tissues following intravenous administration.

**Passive targeting** exploits vascular differences between the target tissue and other tissues to deliver drugs selectively. For example, high molecular mass polymer–drug complexes accumulate in some tumor tissues to a much greater extent than in normal tissues, because the tumor has more permeable capillary beds. Therefore, rather than using lower doses of low molecular mass anticancer drugs, which rapidly pass through all cell membranes and distribute throughout the body, larger and more effective doses of high molecular mass polymer-drug conjugates can be used to target tumors somewhat selectively. In addition, the polymer–drug conjugates can be constructed in such a way as to allow enzymatic cleavage of the drug after the complex has left the bloodstream and been taken up by tumor cells (Fig. 50-1F). In one example of such a system, the anticancer drug **doxorubicin** (see Chapter 32, Pharmacology of DNA Replication and Mitosis) is conjugated to a water-soluble, nonimmunogenic polymer through a petidyl linker. The polymer–drug complex accumulates in mouse melanoma tumors at concentrations up to seventy times greater than in normal tissue because of the relatively leaky microvasculature in the tumor. Once outside the bloodstream and taken up by the tumor cells, lysosomal proteases cleave the peptidyl linker, releasing the cytotoxic drug. The polymer portions either degrade or are eliminated by the kidneys.

In **active targeting,** the polymer–drug conjugate is linked to a molecule that is recognized specifically by cell surface receptors in the tissue of interest. For example, a human IgM antibody directed against a tumor-associated antigen can be used to target a polymer–adriamycin complex to malignant tissues. By linking the drug to the polymer with an acid-labile bond, the adriamycin is selectively released in the acidic environment of the tumor. In another system, galactose is used to target a polymer–drug complex to the liver via the hepatocyte cell surface asialoglycoprotein receptor.

## LIPOSOME-BASED DELIVERY SYSTEMS

Attaching drugs to a single polymer chain creates stable structures that can remain in the circulation for long periods; the drug–polymer complexes discussed above in the context of tissue targeting are examples of such structures. However, these polymer chains can accommodate only small amounts of drug, thus limiting the dose per unit volume administered. The potentially high drug-carrying capacity of **liposomes,** small vesicles with lipid bilayer membranes, makes them an attractive option for a circulating drug delivery system.

Important considerations in the design of liposome-based delivery systems include tissue targeting and protection from the immune system. Highly specific antibodies, analogous to those used for active targeting of polymer–drug complexes, can be used to improve tissue targeting. For example, antibodies against the *Her2* proto-oncogene, implicated in the progression of breast and other cancers, are being explored for tumor targeting. Similarly, antibodies against E-selectin, an endothelial-specific surface molecule, can be used to target vascular endothelial cells. Protection of liposomes from the immune system can be accomplished by the addition of water-soluble polymers to the liposomal surface. As discussed above, moieties such as PEG increase the hydrophilicity of the structures to which they are attached; in this case, liposomes are made more hydrophilic in the blood and, therefore, less prone to uptake by the reticuloendothelial system. Because liposomes with the PEG moiety ("stealth liposomes") have a prolonged circulation time (days), larger doses can be administered without the risk of drug toxicity. These principles have been used to develop liposomes loaded with **daunorubicin** and **doxorubicin** in the treatment of several tumors, including HIV-associated Kaposi's sarcoma. Liposomal **amphotericin B,** used to treat fungal infections, has been approved for clinical application in cancer patients (see Chapter 46, Pharmacology of Fungal Infections). Also, liposomal **cyclosporine** is being studied for targeted immunosuppression following transplant surgery (see Chapter 43, Pharmacology of Immunosuppression).

## Conclusion and Future Directions

The delivery techniques described in this chapter represent selected novel approaches to optimizing absorption, distribution, metabolism, and elimination of drugs. There are several advantages of improved delivery:

- Drug levels can be continuously maintained in a therapeutically desirable range. Sustained release oral formulations, large particles that can be inhaled, and many polymer-based designs have this desirable property.
- Harmful side effects can be reduced by preventing transient high peak blood levels. Altering absorption kinetics, targeted delivery (e.g., antibody labeled polymer–drug complexes), and prevention of first-pass liver metabolism (e.g., transdermal delivery of drugs normally taken orally) achieve this goal.
- The amount of drug required can be reduced, as with advanced inhaler designs. A decrease in the number of required dosages and less invasive administration contribute to improved patient compliance. Mr. F's case illustrates the influence of lifestyle issues on patient compliance.
- Pharmaceuticals with short half-lives, such as peptides and proteins, can be successfully delivered using controlled release polymer-based systems.

Advanced drug delivery technologies also introduce new concerns that must be considered in their design. For example, each material put in the body, as well as its degradation products, must be evaluated for toxic effects; this factor is especially important for synthetic materials such as polymers. Other dangers, such as unwanted rapid release of the drug from a system intended for sustained release, must be avoided. Discomfort caused by the delivery system or its insertion is another potential disadvantage: Mr. F's insulin pump, while providing better control of his diabetes, was uncomfortable to him. Finally, advanced technology is often accompanied by increased cost, which can be a problem for patients, their insurance companies, and hospitals.

Despite these obstacles, advanced drug delivery technologies play an increasingly valuable role in making pharmacologic management of disease safer, more effective, and more agreeable to patients.

## Suggested Readings

Edwards DA, Ben-Jabria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 1998;84:379–385. (*Review of aerodynamic diameter principles and the potential advantages and applications of large, porous inhaled particles.*)

Langer R. New methods of drug delivery. Science 1990;249: 1527–1533. (*Early review, including detailcd descriptions of controlled release systems.*)

Langer R. Drug delivery and targeting. Nature 1998;392:5–10. (*Review of drug delivery techniques, with emphasis on polymer and liposome-based systems as well as novel use of delivery routes.*)

Langer R. Drugs on target. Science 2001;293:58–59. (*Short review that addresses some of the challenges of gene therapy delivery systems.*)

Langer R. Where a pill won't reach. Sci Am 2003;April:50–57. (*Broad overview of concepts in drug delivery.*)

Leong KW, Brott BC, Langer R. Bioerodible polyanhydrides as drug-carrier matrices: I. Characterization, degradation, and release characteristics. J Biomed Mater Res 1985;24:1463–1481. (*Good starting point for learning more about polymer matrix design.*)

Santini JT Jr, Cima MJ, Langer R. A controlled release microchip. Nature 1999;397:335–338. (*More detailed information about intelligent drug delivery using silicon microchips with arrays of drug reservoirs.*)

# IX

# Fundamentals of Drug Evaluation and Pharmacogenomics

# 51

# New Drug Evaluation and Drug Regulation

Patricia Tung and Robert H. Rubin

**Introduction**
**Case**
**Oversight**
  History of the FDA
  Ethics in Clinical Drug Investigation
**Drug Approval**
  Preclinical Evaluation
  Clinical Trials
    *Phase I Studies*
    *Phase II Studies*
    *Phase III Studies*
  FDA Approval
  Special Cases
    *Fast Track Status*
    *Orphan Drugs*
    *Generic Drugs*
  Approval in Other Countries
**Drug Regulation**
  Drug Labeling
  Drug Naming
  Drug Marketing
  Phase IV Studies
  Additional Indications
  Drug Production and Quality Control
  Drug Withdrawals and Recalls
  Drug Costs
  Nonprescription Medications and Supplements
  Drug Patents
**Conclusion and Future Directions**
**Suggested Readings**

## INTRODUCTION

Controlled clinical trials are the scientific and legal basis by which the federal **Food and Drug Administration** (FDA) evaluates new prescription drugs for approval for sale in the United States. Over the last 50 years, improved methods for large-scale clinical studies have precipitated a shift toward evidence-based medicine and helped to accelerate the pace of drug development. In 2001, more than 32 drugs and 8 biologics received approval from the FDA for marketing in the United States. Although this number has varied significantly over the past 5 years, an average of 31 new drugs has been approved each year for the past decade.

Nonetheless, drug discovery and development remains a lengthy, high-risk, and complex process. According to the Pharmaceutical Research and Manufacturers of America, of every five to ten thousand chemically synthesized molecules that are screened, only one becomes an approved drug. This chapter explains the process through which new drugs are evaluated and approved for marketing and sale in the United States. The chapter introduces the organizations involved in drug evaluation and regulation. Next, it describes the clinical research required for approval to market a new drug and each phase of these investigational trials. A short description of the approval process in selected European and Asian countries is also included. The chapter concludes with a discussion of patents, labeling, postapproval studies, and other aspects of drug regulation.

## ■ Case

The history of the discovery of sildenafil citrate as a treatment for erectile dysfunction (ED) provides insight into the drug development and approval process. Throughout the 1980s, Pfizer invested in research and development of a drug intended for the treatment of hypertension and angina. During clinical trials, the hoped-for efficacy was minimal; however, investigators (and subjects) observed that impotent men receiving the treatment were able to achieve erections. Pfizer subsequently patented this molecule for the treatment of ED and proceeded with its development. Between July 1993 and January 1997, twenty-one studies involving 3,000 subjects aged 19–87 were undertaken. In March 1998, Pfizer received approval from the FDA to market sildenafil citrate as an oral therapy for ED. The drug was approved under the trade name Viagra®.

Sildenafil proved successful in treating ED. In addition, the drug offered patients greater convenience over existing treatments, which included an alprostadil pellet inserted into the urethra; an alprostadil injection administered directly into the base of the penis; and a constriction loop designed to slow venous outflow from the penis. Despite its widespread adoption, however, Viagra was linked to a number of deaths. In the 6 months following approval, a period during which more than 6 million prescriptions were dispensed, the FDA received reports of 130 deaths. Of these deaths, 77 were the result of cardiovascular events such as myocardial infarction, cardiac arrest, and coronary artery disease. Subsequent testing revealed conditions, situations, or drug interactions (such as concomitant use of nitrates) that represent contraindications for the use of Viagra. As a result, the FDA ordered Pfizer to amend its label to include expanded warnings of potential side effects and a narrower description of the type of patients for whom Viagra is believed to be safe and appropriate.

## QUESTIONS

1. What ethical standards govern the relationship between physicians and patients in clinical research?
2. What testing must a drug undergo to gain approval for marketing?
3. What regulations exist to ensure drug efficacy and safety?
4. How do these regulations address issues of intellectual property, drug naming, and drug marketing?

## OVERSIGHT

Drug development, testing, and approval is a lengthy process, the major milestones of which are shown in Figure 51-1. Achievement of each of these milestones requires the cooperation of researchers, clinicians, patients, pharmaceutical or biotechnology companies, and the FDA.

## HISTORY OF THE FDA

FDA regulation of drug development evolved in response to key events of the last century and advances in clinical investigation methods. At and before the start of the 20th century, tampering and mislabeling of food and drugs was common. By 1906, public outcry over the unsanitary and unsafe conditions of the meat packing industry—described in Upton Sinclair's *The Jungle*—prompted Congress to pass the **Pure Food and Drug Act.** The Act created the FDA and charged the agency with overseeing these new requirements of the law:

- Drugs must meet published standards of purity and quality.
- Manufacturers must provide correct and truthful labeling for all drugs.
- Interstate and foreign commerce of adulterated and misbranded food and drugs is prohibited.
- Adulteration of food by the removal of valuable constituents, the substitution of ingredients so as to reduce quality, the

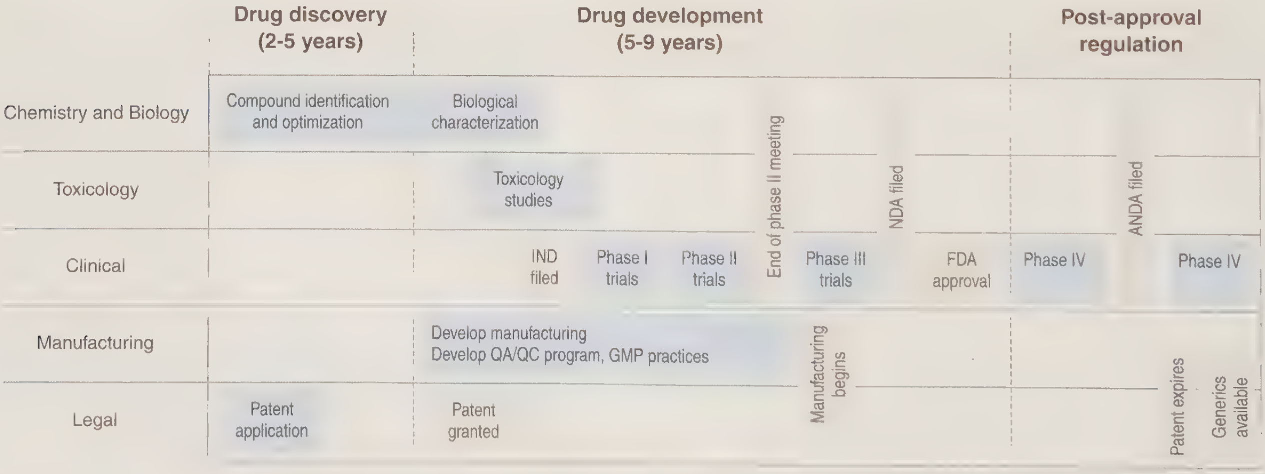

Drug discovery (2-5 years)
Drug development (5-9 years)
Post-approval regulation
Chemistry and Biology
Compound identification and optimization
Biological characterization
Toxicology
Toxicology studies
Clinical
IND filed
Phase I trials
Phase II trials
End of phase II meeting
Phase III trials
NDA filed
FDA approval
Phase IV
ANDA filed
Phase IV
Manufacturing
Develop manufacturing
Develop QA/QC program, GMP practices
Manufacturing begins
Legal
Patent application
Patent granted
Patent expires
Generics available

**Figure 51-1. Life Cycle of Drug Approval.** The life cycle of approval for a new drug is complex, requiring an average of 11 years for completion. Drug discovery, discussed in Chapter 48, produces a new drug molecule. The first patents are usually filed at this stage and are granted several years later. The drug development process requires that biological characterization and toxicology studies in animals are conducted before an Investigational New Drug Application (IND) can be filed. In turn, an IND is required for the start of clinical trials. At the conclusion of successful clinical trials, a drug company files a New Drug Application (NDA), which is reviewed by the FDA. Once a drug is approved, it must be monitored for safety for the remainder of its lifespan (so-called Phase IV). The first of the drug's patents expire 20 years after its application. Abbreviated New Drug Applications (ANDAs) can be filed before the original patent expires. Once a patent expires, generic versions of that drug can become available.

addition of deleterious ingredients, and the use of spoiled animal and vegetable products is prohibited.

In 1938, more than 100 Americans—many of them children—died after consuming "Strep-Elixir," an untested product containing a sulfonamide and a chemical analogue of antifreeze, diethyleneglycol. In response, Congress mandated that manufacturers obtain premarket approval from the FDA contingent on demonstrated safety.

1962 marked the next major change in FDA regulations, when legal modifications were made to require proof of a drug's efficacy as a condition for approval. Table 51-1 provides a chronology of major legislation affecting the scope and direction of the FDA's oversight of drug approval.

## ETHICS IN CLINICAL DRUG INVESTIGATION

The FDA has codified standards of ethical behavior for all parties involved in clinical research—clinicians, pharmaceutical companies, and medical institutions. The ethical relationship between physicians and patients is governed by the notion that clinical trial research represents a partnership between investigator and subject. Four major ethical principles, established by the **International Conference on Harmonization** and the **Declaration of Helsinki,** support this partnership. These principles are as follows:

- The trial must minimize the risks for participants.
- Provisions must be made for the overall care of the patient.
- The investigator is responsible for terminating the trial when the risks become incompatible with the goals of the trial.
- Adverse events must be reported immediately to an ethics or safety committee.

Furthermore, investigators must ensure the fair and equitable choosing of subjects by limiting the selection of study patients to those with conditions that may benefit from the drug in question. This is done to balance the potential risks, many of which are unknown, with the potential benefits, the type and extent of which are also unknown.

In addition, investigators must obtain subjects' **informed consent.** Informed consent is not just a signed document, but instead a process in which patients are made aware of the potential risks and benefits of the trial and must make an informed decision to participate voluntarily in a clinical study. For patients with poor prognoses, informed consent encompasses the understanding that the research likely will not benefit them, but may benefit future patients.

At the institutional level, the FDA relies on independent **Institutional Review Boards** (IRBs) or **Independent Ethics Committees** (IECs) to ensure the rights and welfare of those participating in clinical trials. FDA regulations mandate that clinical study protocols be reviewed for legal and ethical issues by an IRB/IEC. These regulations give IRBs/IECs the authority to approve, require modification of, or disapprove human subject research. Specifically, the IRB/IEC must determine whether the proposed research:

- minimizes potential risk to human subjects;
- poses risks that are reasonable relative to the anticipated benefit and potential scientific gain of the research;
- includes equitable selection of subjects;
- provides for an effective informed consent process; and
- contains safeguards for vulnerable populations, such as children and the mentally disabled.

IRB oversight and approval begins before the commencement of human trials and continues for the duration of clinical trials. IRB/IEC membership comprises five or more experts and lay

**TABLE 51-1** Major Legislation Affecting FDA Regulation

| LEGISLATION | OUTCOME |
|---|---|
| Pure Food and Drug Act of 1906 | Requires truthful labeling for all drugs |
| 1912 Amendment to the Pure Food and Drug Act | Prohibits fraudulent advertising claims |
| Food, Drug and Cosmetic Act of 1938 | Requires proof of a drug's safety and purity |
| Durham–Humphrey Act of 1952 | Grants FDA authority to determine which drugs may be sold without a prescription |
| 1962 Kefauver–Harris Amendments to the Food, Drug and Cosmetic Act | Requires proof of efficacy as well as safety for new drugs and drugs approved since 1938; also establishes guidelines for adverse event reporting, clinical testing, and advertising |
| Orphan Drug Amendments of 1983 | Provides incentives to manufacturers of drugs that treat orphan diseases |
| Drug Price Competition and Patent Restoration Act (Hatch–Waxman Act) of 1984 | Abbreviates and modifies New Drug Applications (NDAs) for generic drugs. Creates patent life extensions for delay caused by FDA review. Extensions limited to 5 extra years or 14 years post-NDA approval |
| Expedited Drug Approval Act of 1992 | Allows accelerated FDA approval for drugs of significant medical need, but requires detailed postmarketing surveillance |

persons from various backgrounds. Federal regulations stipulate that IRB membership must contain at least one member whose primary expertise is in a scientific area, one member whose primary expertise is in a nonscientific area, and one member who is not affiliated with the institution overseeing the clinical research protocol. In addition, the other members' qualifications must be such that the IRB is able to evaluate research proposals in terms of institutional requirements, applicable law, standards of professional practice, and community attitudes. Thus, many IRBs include clergy, social workers, and attorneys.

## DRUG APPROVAL

The investigation of a new drug comprises several phases, beginning with preclinical evaluation and proceeding through phase III studies, after which the FDA may consider a new drug for approval.

### PRECLINICAL EVALUATION

**Preclinical research** is used to establish potential efficacy and safety of a compound for use in human trials. During this stage of testing, described in Chapter 48, Drug Discovery and Development, a compound is studied to determine its biologic actions, chemical properties, metabolism, and a process for synthesis and purification. The two principal means of preclinical evaluation are in vitro assays and in vivo laboratory animal testing.

Animal testing occurs in **short- and long-term** phases. **Short-term testing** can range from 2 weeks to 3 months or longer, and focuses on the absorption, distribution, metabolism, and excretion of the compound, as well as on evaluating toxicity. Quantitative estimates of toxicity, such as the **no-effect dose,** the **minimum lethal dose,** and the **median lethal dose** are determined at this stage. Preclinical testing provides critical information about the threshold level of safety required to proceed with human studies and remains an important decision point for proceeding with drug development. Many potential drug candidates fail toxicology studies or other aspects of preclinical investigation and do not proceed to human trials.

*Before proceeding to clinical studies, a sponsor must provide evidence that a drug is reasonably safe for investigation in humans.* If the compound of interest has been previously marketed or studied, the sponsor has several options for fulfilling this requirement:

- compile existing data from previous in vitro laboratory and animal testing;
- compile existing data from previous clinical testing in relevant populations; or
- undertake a new preclinical development program.

The vehicle for submitting these data to the FDA is the **Investigational New Drug** application (IND). The IND contains data from the preclinical development, the proposed protocol for human trials, and other background information. It also contains information relevant to the composition and stability of the drug and evidence that it can be manufactured in consistent batches both for clinical trials and for commercial use. The IND does not grant a manufacturer permission to market a drug. Rather, it grants an exemption from a federal law that prohibits unapproved drugs from being shipped in interstate commerce; this exemption is a necessary part of multicenter clinical investigation. Commercial INDs are submitted by sponsors with the ultimate goal of obtaining approval for marketing and sale of a new drug product. Noncommercial filings, such as **Investigator, Emergency Use,** and **Treatment** INDs, are used for different purposes, as described later in this chapter.

The FDA must review the IND within 30 days and decide if human trials may begin. Figure 51-2 is a flowchart representing the process the FDA uses to review an IND. The areas

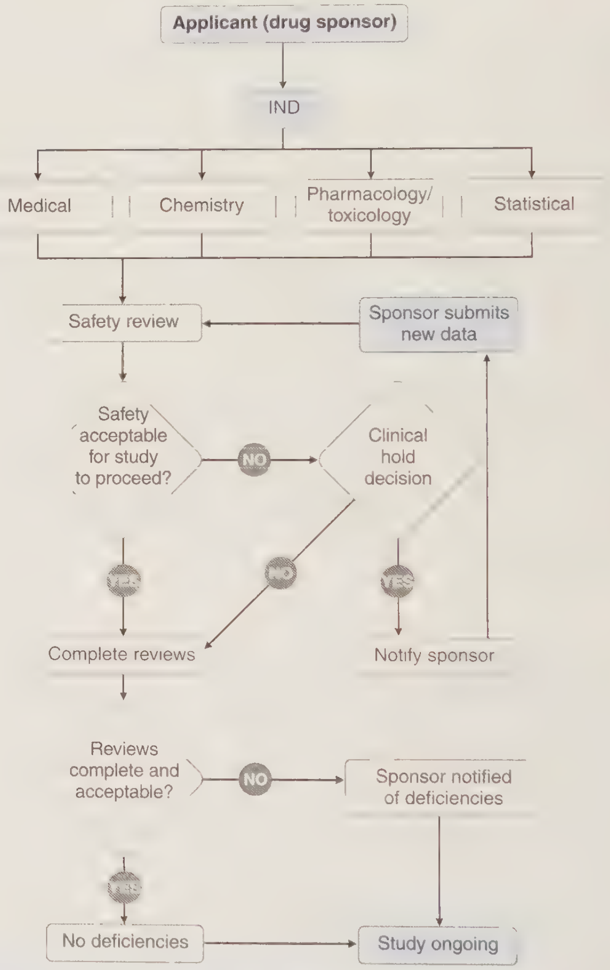


**Figure 51-2. Process of Investigational New Drug Review.** When an investigational new drug application (IND) is filed, the FDA has 30 days to review the application. This flow diagram shows the process of internal review that is undertaken by the FDA. The drug sponsor provides medical, chemical, pharmacology/toxicology, and statistical data for the compound that are reviewed by separate committees within the FDA. If the safety of the compound is deemed acceptable, an IND is approved once the application is complete. If the safety of the compound is deemed unacceptable for clinical trials or more data are needed, the sponsor is given the opportunity to submit new results from further testing. In some cases, a study is allowed to proceed while the sponsor is answering any deficiencies (not shown). Blue boxes correspond to actions by the drug sponsor; white boxes correspond to actions by the FDA.

of review include a **medical review, chemistry review,** and **pharmacology/toxicology review.** The medical review evaluates the clinical trial protocol to ensure that participants will not be exposed to undue risk. It also reviews the protocol to determine whether the studies are capable of yielding data sufficiently robust to support subsequent clinical trials and eventual FDA approval. The medical review is performed almost exclusively by FDA physicians. The chemistry review evaluates the stability of a drug and the reproducibility of its synthesis and purification. In particular, evaluators review any chemistry or manufacturing differences between the proposed clinical use and the product used in animal toxicology trials. The pharmacology/toxicology review evaluates the safety of the drug, based on an integrated summary of toxicologic and pharmacologic effects in animals and in vitro.

If the IND review reveals the potential for unreasonable risk to participants, the FDA contacts the sponsor and a **clinical hold** is issued preventing initiation of clinical studies. The sponsor must address any issues in question before the clinical hold is lifted. If it is found that a sponsor did not accurately disclose the risk of the study to investigators, the FDA may impose a clinical hold at any phase of clinical trials.

## CLINICAL TRIALS

Once an IRB approves a study protocol, clinical studies proceed in three phases (see Table 51-2). Trial protocols must be structured to provide reliable answers to these questions, and must consider the following:

- which prospectively defined outcome variables are feasible to measure and are scientifically valid;
- whether a control group is possible and what treatments, if any, need to be used in control group subjects;
- the ease with which subjects and investigators may be blinded;
- the definition and scope of disease; and
- the number of participating trial sites and patients

Study investigators must assess chance, bias, and confounding factors that affect all trials, and incorporate measures to address these problems. **Subject bias** can be countered with provision of a placebo, an inert substance with the same appearance as the drug under observation. **Observer bias** can be countered by blinding, usually by coding the drug and placebo, so that their identities are masked, and the investigators cannot "see" which subjects are being administered which treatment. When the identity of the intervention is unknown to both subject and observer, the study is a **double blind** study.

The natural fluctuation and spontaneous remission of many diseases also confounds clinical trials. A **crossover design,** in which each study group is given the test drug alternately with placebo, can protect against the misinterpretation of the natural fluctuations of disease. The presence of other diseases or risk factors, known or unknown, represents a third major confounder of clinical trials. Careful medical histories and **randomization of subjects** can counter some of the effects of these risk factors. In addition to the strategies mentioned above—use of placebos, blinded studies, and crossover regimens, and randomization—a large sample size helps to minimize the effect of these factors. It is important to note that *generally only phase III trials are randomized, placebo controlled, double blind studies.*

Table 51-2 summarizes the number of patients, length of time required, and purpose of each stage of clinical trials.

**TABLE 51-2** Clinical Drug Testing in Humans

| | NUMBER OF PATIENTS | LENGTH OF PHASE | PURPOSE |
|---|---|---|---|
| Phase I | 20–100 | Several months | Mainly safety |
| Phase II | Up to several hundred | Several months to 2 years | Effectiveness and short-term safety |
| Phase III | Several hundred to several thousand | 1–4 years | Safety, dosage, effectiveness |

### Phase I Studies

**Phase I studies** generally involve between 20 and 100 healthy subjects and are intended to establish the safety and tolerability of a drug. If high levels of toxicity are expected, such as with many cancer drugs, patients with the target condition may be used in place of healthy volunteers. The focus of investigation is the drug's overall effect in the body, including **maximum tolerated dose,** absorption, distribution, metabolism, and excretion. To determine the effect of varying doses, subjects are started on a dose anticipated to have little effect, and receive increasing doses thereafter.

The primary goal of phase I studies is to establish safety, toxicity, and side effects. Phase I studies often involve **non-blinded trials,** in which subject and investigator both are aware of what is being administered. Phase I studies must yield sufficient information about a drug's pharmacokinetic effects to inform the design of scientifically valid phase II studies. For example, knowing the drug's clearance enables study designers to determine dosing frequency in later study phases.

### Phase II Studies

**Phase II studies** involve up to several hundred patients with the medical condition of interest. Phase II clinical trials have multiple objectives, including acquisition of preliminary data regarding the effectiveness of the drug for a particular condition. Like phase I trials, phase II trials continue to monitor safety. Because phase II trials enroll more patients, they are capable of detecting less common adverse events. Phase II studies also evaluate **dose–response** and dosing regimens, which are critically important in establishing the optimum dose or doses and frequency of administration.

A typical phase II design may involve a **single-blind trial** in which the drug of interest is evaluated against placebo and/or an existing therapy. The trial usually compares several dosing options to obtain optimum dose range and toxicity information. The results of phase II studies are critically important in establishing a specific protocol for phase III studies. Phase II results can also be used to pinpoint additional data that must be collected in phase III trials, such as monitoring liver function tests if phase II data suggest possible hepatotoxicity.

## Phase III Studies

**Phase III studies** involve several hundred to several thousand patients and are conducted at multiple sites and in settings similar to those in which the drug ultimately would be used. Phase III studies are based on specific **clinical endpoints** (also known as **primary endpoints**) or **surrogate endpoints** (also known as **secondary endpoints**). Primary endpoints include survival, improvements in patient functioning, or improvements in how patients feel. Surrogate endpoints are markers for decreased disease burden, such as the reduction of serum levels of biochemical markers (e.g., glucose or LDL cholesterol), increases in cardiac output, or the reduction in size of a tumor. *Although surrogate endpoints are usually easier to measure, a drug's approval usually depends on demonstrating effectiveness in improving primary endpoints.*

To distill true effects from placebo effects and from natural fluctuations in the course of disease, phase III studies typically employ randomized, controlled, double-blind trials with multiple study arms. Because of the large number of patients under study, phase III results typically provide an adequate basis for extrapolating results to the general population.

Before the start of phase III trials, the sponsor and the FDA conduct **"End of Phase II" meetings.** The purpose of this meeting is to establish the safety of proceeding with phase III studies and to cement study objectives and designs. The FDA encourages sponsors to submit data supporting the indication for the drug, chemistry data, animal data, and proposed additional data, results of phase I and II studies, statistical methods used, protocols for phase III studies, and proposed labeling for the drug, before the "End of Phase II" meeting.

The FDA requires satisfactory compliance with specific endpoints, often determined by the FDA or in conjunction with the drug's sponsor. If the results of phase I and II studies do not satisfy these requirements, the FDA can mandate additional studies or issue a clinical hold before allowing phase III investigation to begin.

*Although the drug investigation process aims to prevent undue risk to human participants and ultimately, to all patients, it is impossible to predict all adverse events associated with a particular drug.* At the time of FDA approval, the safety and effectiveness of a new drug is usually evaluated in several thousand individuals. This may represent a small fraction of the population who may take this drug once it is available commercially. For this reason, the FDA and sponsors maintain surveillance of a drug's safety and effectiveness during and after market launch.

# FDA APPROVAL

Approval and regulation of new drugs is based on the **New Drug Application** (NDA). The NDA must contain all relevant data that a sponsor has collected during a drug's research and development. As such, the data gathered for the IND are integrated into the NDA. The components are common across all NDAs filed, and the FDA mandates that the NDA contain the following sections: index, summary, chemistry, manufacturing and quality control, samples, methods validation package and labeling, nonclinical pharmacology and toxicology, human pharmacokinetics, metabolism and bioavailability, microbiology, clinical data, safety update report (typically submitted 120 days after NDA submission), statistical information, case report tabulations, case report forms, patent information, patent certification, and other information. NDA submissions typically run for thousands of pages.

Upon receipt of an NDA, two decisions are made. First, the FDA classifies the application into one of several categories, based on whether the drug is a new molecular entity or a duplication of an already marketed product. All new chemical entities proposed to be administered to patients chronically (longer than 6 months) are tested for carcinogenesis in 2-year studies in two species of laboratory animals (usually rat and mouse). The NDA is then routed to a specific review committee based on the proposed indication of the drug. The FDA next determines whether the NDA will receive a priority or standard review. A priority review is granted based on the existence of medical need and the absence of prior marketed therapies that have similar therapeutic qualities. At that point the NDA undergoes a preliminary review to assess the completeness of the information submitted.

For an NDA undergoing standard review, the FDA will first evaluate the application to ensure that the sponsor has included evidence of the drug's proposed mechanism of action based on in vitro proof of concept and animal testing. The agency also will review the study protocol's inclusion and exclusion criteria to determine if study subjects meet these criteria, and if the criteria ultimately define the likely treatment population for the drug. The FDA also will examine dosimetry, pharmacokinetic, pharmacodynamic, and metabolism data. Last, the sponsor must include toxicology information and duration of the drug in the body. The FDA organizes this review into five or six categories: **medical review, biopharmaceutical review, statistical review, pharmacology review, chemistry review,** and **microbiology review** (required only for antibiotics). Figure 51-3 is a flowchart representing the process that the FDA uses to evaluate an NDA.

In addition to reviews internal to the Agency, the FDA may also call on **external advisory committees** for input on NDAs. These committees provide non-FDA (usually academic but also political) input and allow for consultation with experts in a particular field. Although the FDA usually incorporates advisory committee recommendations into its decisions, these opinions are not binding.

Following NDA submission, the FDA maintains ongoing communication with the sponsor regarding scientific or other issues that arise during review. Meetings are held between the sponsor and the agency, particularly if additional data are needed. A sponsor may submit additional data or a new analysis of previously available data after submission of the NDA. However, this is considered an amendment of the NDA and can result in delayed approval times.

The FDA may take three possible actions on an NDA—an NDA may be **approved, not approved,** or ruled **"approvable."** If the Agency deems an NDA not approved or approv-

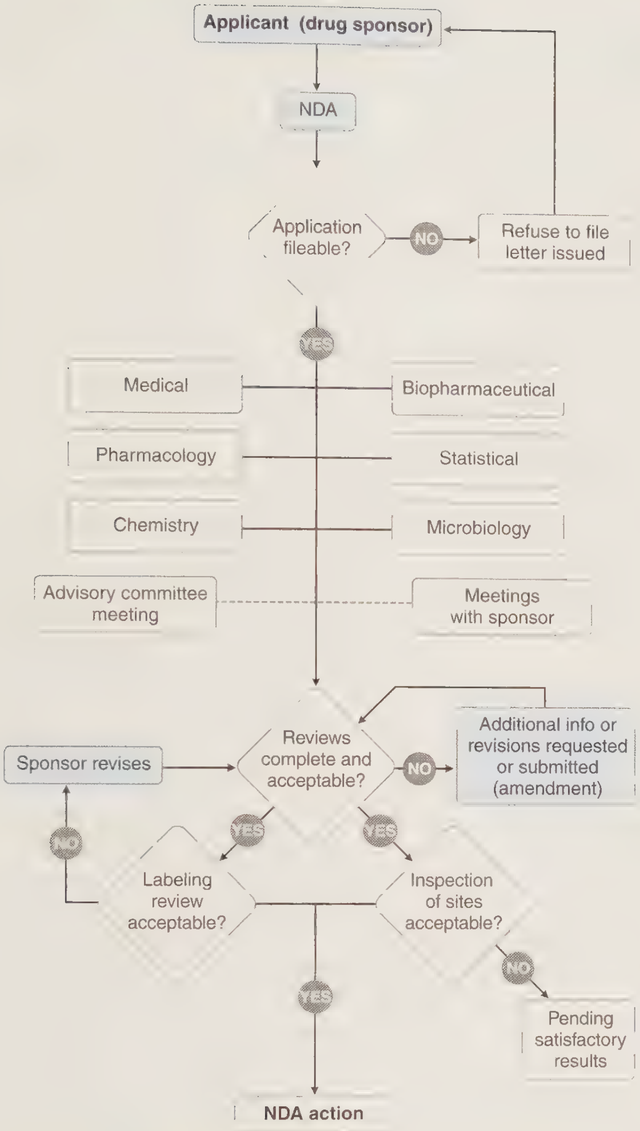

Applicant (drug sponsor)
NDA
Application fileable?
NO
Refuse to file letter issued
YES
Medical
Biopharmaceutical
Pharmacology
Statistical
Chemistry
Microbiology
Advisory committee meeting
Meetings with sponsor
Sponsor revises
Reviews complete and acceptable?
NO
Additional info or revisions requested or submitted (amendment)
NO
YES
YES
Labeling review acceptable?
Inspection of sites acceptable?
NO
YES
Pending satisfactory results
NDA action

**Figure 51-3. Process of New Drug Application Review.** When a new drug application (NDA) is filed, the drug sponsor provides data regarding the drug's medical, pharmacologic, chemical, biopharmaceutical, statistical, and microbiologic characteristics that are reviewed by separate committees at the FDA. The FDA or an FDA Advisory Committee (optional) may meet with the sponsor. If the review is complete and acceptable, then the drug application is reviewed for acceptable labeling (official instructions for use). The manufacturing sites and sites where significant clinical trials are performed also undergo review. Blue boxes correspond to actions by the drug sponsor; white boxes correspond to actions by the FDA.

able, it must list the deficiencies in the application and provide suggested changes. Often the FDA will meet with the sponsor to discuss what steps must be taken to secure approval. Although "not approved" applications may require significant new studies to be undertaken, they are frequently abandoned. "Approvable" applications usually need relatively minor modifications to the NDA or more supporting data.

In general, of every 100 INDs submitted, 70 will complete phase I trials and proceed to phase II; approximately 33 of the original 100 will complete phase II and proceed to phase III; 25 to 30 of the original 100 will complete phase III; and finally, 20 of the original 100 will ultimately be approved for marketing.

## SPECIAL CASES

### Fast Track Status

**Accelerated development and approval** status, or **"fast track"** status, may be given to products that demonstrate the potential to fulfill an unmet medical need for a serious or life-threatening disease. For a drug with this status, approval may be granted based on evaluation of surrogate endpoints. An accelerated review period for such an NDA may be only 6 months, as compared to the standard review time of 10 to 12 months. As a condition of expedited review and approval, the FDA may require the sponsor to conduct thorough **post-marketing surveillance** to assess clinical benefit and safety. If a clinical benefit or safety are not found, the FDA may withdraw the approval without the process it would follow for drugs that had received standard approval.

### Orphan Drugs

The FDA has specific regulations for the development and approval of drugs for orphan diseases, defined as diseases that affect fewer than 200,000 individuals in the United States. Absent additional incentives, pharmaceutical companies would typically be disinterested in developing products for such small markets. In an attempt to encourage development of drugs for rare diseases, Congress passed the **Orphan Drug Act** in 1983. The legislation offers generous financial incentives to companies that develop orphan drugs. In addition, an orphan drug enjoys exclusive approval for the orphan indication for 7 years following approval. Since 1983, the FDA has approved more than 180 such products:

- Infliximab—the first approved treatment for Crohn's disease
- Thalidomide—for an inflammatory symptom in Hansen's disease (leprosy)
- Denileukin diftitox—for cutaneous T-cell lymphoma following failure of other treatments
- Atovaquone—for *Pneumocystis carinii* pneumonia

To expand access to investigational drugs, the FDA has created **compassionate use protocols,** which permit promising investigational therapies to be used, before general approval, for extremely sick patients who are not eligible for an ongoing clinical trial. To be eligible for a compassionate use protocol, also known as a treatment investigational new drug (**treatment IND**), the drug must show preliminary evidence of efficacy, patients must be likely to die or suffer rapid disease progression within several months, or to die prematurely without treatment, and there must be no comparable therapy to treat the disease at that stage.

### Generic Drugs

The FDA also oversees approval of **generic drugs.** The agency defines a generic drug as comparable to an innovator drug in dosage form, safety, strength, route of administration, quality, performance characteristics, and intended use. Under the **Drug Price Competition and Patent Term Restoration Act of**

**1984,** also known as the **Hatch–Waxman Act,** a company may submit an **Abbreviated New Drug Application** (ANDA) before the patent governing the brand name drug expires. However, the company must wait for the original drug's patent to expire before it can market a generic version. The first company to file an ANDA has the exclusive right to market the generic drug for 180 days.

ANDAs for generic drugs are not required to provide data establishing safety and efficacy, because this has been established in the NDA for the innovator drug. To establish **bioequivalence,** which is required in the ANDA, sponsors may submit a formulation comparison, comparative dissolution testing where there is a known correlation between in vitro and in vivo effects, in vivo bioequivalence testing comparing rate and extent of absorption of the generic to the reference product, and, for nonclassically absorbed products, a head-to-head evaluation of comparative effectiveness based on clinical endpoints.

In addition, an ANDA sponsor must provide evidence that its manufacturing processes and facilities, as well as any outside testing or packaging facilities, are in compliance with federal **Good Manufacturing Practice** regulations.

## APPROVAL IN OTHER COUNTRIES

Drug regulation and approval varies by country. In Canada, **Health Canada** administers the regulations embodied in the **Canadian Food and Drugs Act.** According to these regulations, an evaluation of safety, purity, effectiveness, potency, and stability must be undertaken. In addition, scientific data must be provided to show safety and efficacy when used in accordance with the label. Although the regulations do not specify the types of studies to be completed, the standard submission for any new drug generally includes acute, subacute and chronic studies, two-generation reproduction studies, and teratology studies.

Before drugs may be sold in EU countries, they must first be evaluated by the **European Medicines Evaluation Agency** and approved by the **European Union.** The EU may impose specific terms on a drug as a condition of approval. These may be the same as or different from the requirements of the U.S. FDA. For example, Viagra® was approved in the EU for a period of 5 years, after which it is to be evaluated to determine whether it may be sold over the counter.

In Switzerland, a new drug application is submitted to the **Interkantonale Kontrollstelle Für Heilmittel** (IKS) upon completion of clinical trials. The IKS registers pharmaceuticals and classifies them on a scale from A to E. Drugs with an A through C classification may only be sold by a pharmacist, while D and E drugs may be sold at nonpharmacy drug stores. Once approval is granted by the IKS, a manufacturer must apply for a price to sell the drug as well as a spot on the **"sick fund"** list of reimbursable products.

In Japan, approval of new drugs is granted by the **Ministry of Health and Welfare** and represents a decision that a drug is both safe and effective. The Ministry's decision is based on an evaluation by 15 members of the **Central Pharmaceutical Affairs Council** who evaluate scientific data, including data on the effectiveness of the drug in people of Japanese origin. In addition, the Ministry must determine that a manufacturer's facilities and equipment meet regulatory standards before a drug may be sold.

## DRUG REGULATION

In the United States, FDA regulation and oversight continues after a drug receives approval. Drug manufacturers must adhere to federal rules in their labeling and marketing activities for the life of a drug.

### DRUG LABELING

The FDA has established a standard format and organization for labeling an approved drug. A drug label must contain a description of the drug that includes proprietary and chemical name, formula and ingredients, clinical pharmacology, indications and usage, contraindications, warnings, precautions, adverse reactions, drug abuse/dependence, overdosage, dosage, rate of administration, and how the drug is supplied. This information is also known as the drug's **package insert.** When a new drug approaches approval, the FDA reviews and negotiates a sponsor's draft package insert to ensure that the labeling is justified by the data submitted in the NDA. Figure 51-4 displays a sample package insert for an approved drug.

### DRUG NAMING

Another facet of drug approval involves determination of a drug's name. A drug has two principal names by which it is known, the **generic name** and the **brand name** or **trade name.** A drug's generic name is based on its chemical name, and is unprotected by a trademark. However, the generic name must be approved and registered with the **U.S. Patent and Trademark Office** (PTO). For example, sildenafil citrate is the generic name by which Viagra® is known. In contrast, a drug's brand name refers to the exclusive name of a substance or drug product owned by a company under trademark law, regardless of whether the name has been registered with the PTO. A brand name need not be approved, provided it does not infringe on an existing trademark. Viagra® is the trade name for sildenafil citrate.

### DRUG MARKETING

A drug's sponsor or manufacturer must submit all marketing materials to the FDA for review before public dissemination. All promotional materials are subject to the same restrictions as the product labeling. As such, these materials are evaluated to ensure that they are accompanied by a package insert that contains the information required by FDA labeling requirements.

The FDA continues to review advertising material intended for clinical audiences and the general public even after a drug has been approved. The agency attempts to ensure that adverse events and side effect information are specifically mentioned and accurately communicated.

Advertising aimed at consumers must specifically mention adverse effects and warnings, and must be clearly communicated. Clearly, though, surveillance of all consumer-focused advertising is more difficult because it includes television, radio, and difficult-to-control internet outlets. The FDA will call on a drug company to modify or remove an advertisement

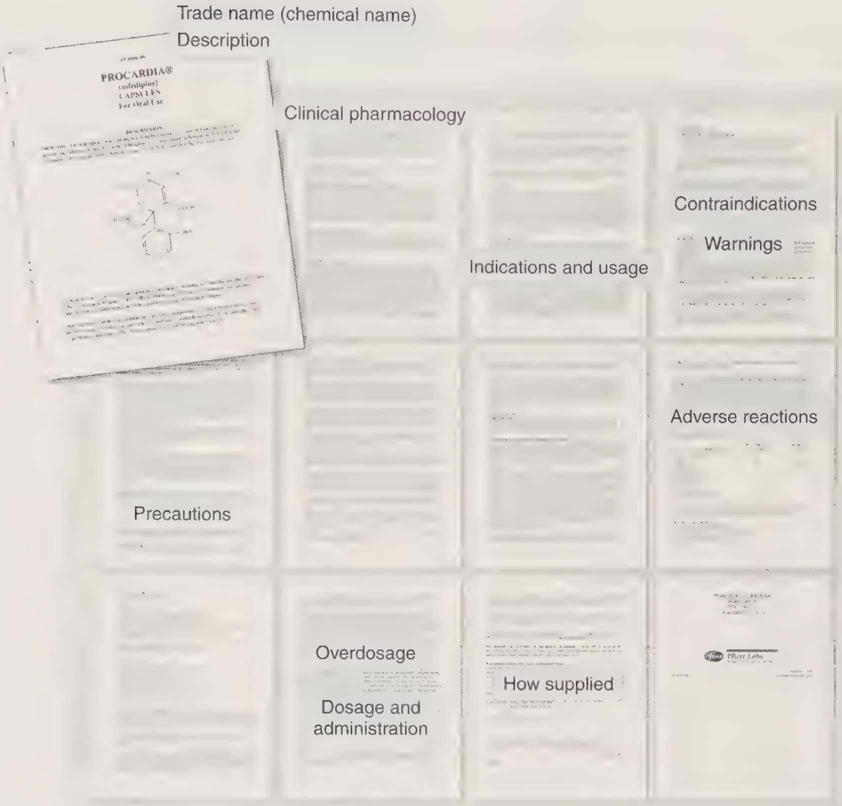

Trade name (chemical name)
Description
PROCARDIA®
Clinical pharmacology
Contraindications
Warnings
Indications and usage
Adverse reactions
Precautions
Overdosage
Dosage and administration
How supplied

**Figure 51-4. Sample Drug Package Insert.** The package insert contains several mandated sections, as highlighted. These sections include the trade name and chemical name, description (which frequently contains the molecular structure), clinical pharmacology, indications and usage, contraindications, warnings, precautions, adverse reactions, drug abuse/dependence (not shown for this drug), overdosage, dosage and administration, and how the drug is supplied (e.g., tablet, liquid, etc.).

that makes unsubstantiated claims, or fails to inform patients and providers of possible adverse effects. Fines may be levied for noncompliance.

## PHASE IV STUDIES

Once approved, a drug enters **phase IV,** which lasts for the market life of the drug. The FDA may require the sponsor to conduct specific types of Phase IV studies, to evaluate the safety of the drug when administered to large populations outside controlled settings. Because rare adverse reactions are more likely to be detected when large numbers of patients are exposed to a drug after it has been approved, the wider use that occurs postapproval expands the known safety information about a drug. For example, adverse reactions that occur in fewer than 1 in 3,000 to 5,000 patients are unlikely to be detected during clinical trials. Table 51-3 illustrates the number of patients needed to detect rare adverse events.

The FDA requires sponsors to engage in ongoing reporting of adverse events postapproval. The agency also collects adverse event information from physicians and health professionals through the **MEDWatch** program. MEDWatch is a centralized gateway through which physicians and health professionals may report or obtain information on the safety of prescription and over-the-counter drugs, biologics, medical and radiation-emitting devices, and, special nutritional products (e.g., medical foods, dietary supplements and infant formulas). The FDA collects information from these and other sources in a comprehensive database, known as the **Adverse Events Reporting System** (AERS). The database is reviewed to detect serious reactions that were previously unknown or that do not appear in a drug's package insert. Often these are rare adverse events that emerge once the drug is used in larger populations.

For example, in November 2002, the FDA required that MedImmune, Inc. issue a safety alert regarding palivizumab, based on adverse events reported in the AERS database. Although there had been no cases of anaphylaxis by the time of approval, the nature of the drug (it is a humanized mouse anti-RSV monoclonal antibody) led MedImmune to present anaphylaxis as a theoretical risk. Since then, two patients with anaphylaxis have been reported after 4 years of worldwide marketing and a review of more than 400,000 patients and 2,000,000 doses administered. Consequently, MedImmune, Inc. revised the Warnings, Overdosage, and Post-Marketing Experience sections of its prescribing information brochure to reflect the small but apparently real risk of anaphylaxis.

## ADDITIONAL INDICATIONS

Once a drug is approved, physicians are permitted to prescribe the drug in various doses or dosage regimens, and for additional clinical indications, known as **"off-label" use.** They may also conduct investigational studies with the drug, provided they follow the rules of informed consent and have IRB approval. Though physicians are permitted to use a drug off-label, they may nonetheless be liable for medical malpractice, just as they might be for any other treatment decisions. Pharmaceutical companies, however, may not market the drug for any indications other than those for which it has been approved. Current regulations prohibit pharmaceutical

TABLE 51-3 Sample Size as a Function of Adverse Effect Frequency

| FREQUENCY OF EVENT IN CONTROLS | SAMPLE CONDITION | NUMBER OF SUBJECTS REQUIRED |
|---|---|---|
| 1/100 | Any congenital heart defect | 1800 |
| 1/1000 | Facial clefts | 18,000 |
| 1/10,000 | Tricuspid atresia | 180,000 |
| 1/100,000 | Myocardial infarction | 1,800,000 |

companies from providing any marketing materials, including scientific articles, on the off-label use of a drug if a physician does not request it. To market a drug for a new indication, a pharmaceutical company would have to seek and obtain an additional NDA.

## DRUG PRODUCTION AND QUALITY CONTROL

In addition to demonstrating a drug's safety and efficacy, manufacturers also must comply with FDA regulations for manufacturing as a requirement for drug approval. These **"Good Manufacturing Practice"** (GMP) guidelines govern quality management and control for all aspects of drug manufacturing, and the FDA makes unannounced inspections of manufacturing facilities to determine compliance. FDA regulations specify impurity tolerance levels, quality control procedures, and testing of sample batches.

A company must obtain prior FDA approval before implementing any manufacturing change determined by FDA to have substantial potential to affect the identity, strength, quality, purity, and potency of a drug as they relate to safety or effectiveness. Other changes may be implemented either with or without submission of a supplemental NDA. Those changes not requiring a supplement may be reported in the annual report filed with the FDA or on another date determined by the Agency.

## DRUG WITHDRAWALS AND RECALLS

If a drug is found to cause unacceptable toxicity or new adverse reactions, the FDA may request or impose a **drug withdrawal,** whereupon the manufacturer agrees to cease marketing the drug in the United States. For example, in August 2001, Bayer announced that it would withdraw Baycol (cerivastatin) from the U.S. market because postmarketing surveillance data showed that it could cause potentially fatal rhabdomyolysis. In 1997, Wyeth–Ayerst Laboratories announced that it would withdraw the diet drug Redux (desfenfluramine) because of the risk of heart valve abnormalities. From 1997 to 2001, the FDA requested 13 drug withdrawals. Most were for recently approved drugs, but three of the withdrawals were for drugs approved before 1990.

A **drug recall** is a temporary removal of a drug from market, and usually occurs when, upon postapproval inspection, the FDA encounters a manufacturing site that does not meet the GMP standards. Drug recalls also may be initiated by the manufacturer, and may range from Class I to Class III, based on the severity and likelihood of adverse health consequences (see Table 51-4). The FDA may also order a **market withdrawal,** which temporarily pulls the drug from market, when minor violations occur that would not be subject to FDA enforcement action, such as the postdistribution tampering that occurred in the 1980s with the Tylenol™ brand of acetaminophen.

## DRUG COSTS

Many factors affect the price of a drug. Obvious drivers of price include the research and development costs that have been incurred and the cost of competing therapies. A complicated manufacturing process may also contribute to higher drug prices. Manufacturers also consider the extent of coverage and reimbursement for the drug by third-party insurers. For example, manufacturers' pricing decisions may be affected if the target population is insured primarily by insur-

TABLE 51-4 Classes of Drug Recalls

| RECALL TYPE | REASON |
|---|---|
| Class I | Reasonable probability that use of or exposure to the product will cause serious adverse health consequences or death. Example: microbial contamination of an injectable drug. |
| Class II | Use of or exposure to the product will cause temporary or medically reversible adverse health consequences. Probability of serious adverse health consequences is remote. |
| Class III | Use of or exposure to the product is not likely to cause adverse health consequences. Example: a quantity packaging error. |

ers with policies that mandate alternatives to or controls on drugs above a particular price level. In addition, manufacturers factor a desired profit margin into a drug's ultimate price. During the life of the patent, a company has a monopoly on a given drug, which allows for few price controls. However, once the patent expires, competition from generic manufacturers may substantially reduce the price of the drug.

In many foreign countries (especially those with government-sponsored health insurance), the cost of the drug may be dictated by the government or negotiated between the drug company and the government. A drug's price in other countries may, therefore, be considerably lower than the cost of the drug in the United States.

## NONPRESCRIPTION MEDICATIONS AND SUPPLEMENTS

The 1951 **Durham–Humphrey Amendment** to the Food, Drug and Cosmetic Act defined prescription drugs as those unsafe for use except under professional supervision. In determining which drugs do not require a prescription for dispensation, the FDA examines a drug's toxicity and the facility with which a condition may be self-diagnosed. Because OTC drugs are sold in lower doses than their prescription counterparts, and are used primarily to treat symptoms of disease, the FDA requires their labels to contain the following:

- intended uses of the product as well as the product's effects;
- adequate directions for use; and
- warnings against unsafe use, side effects, and adverse reactions

Although OTC products present a danger of misuse or misdiagnosis in the absence of physician oversight, the increasing availability of these products has provided many U.S. citizens with access to effective and relatively inexpensive treatments.

The **Dietary Supplement Health and Education Act** of 1994 defines **dietary supplements** as any product intended for ingestion as a supplement to the diet, including vitamins, minerals, herbs, botanicals, other plant-derived substances, amino acids, concentrates, metabolites, and constituents and extracts of these substances. The FDA oversees the safety, manufacturing, and health claims made by dietary supplements. The FDA does not, however, evaluate the efficacy of supplements as it does for drugs. The FDA may restrict or halt the sale of unsafe supplements, but it must demonstrate that those supplements are unsafe before taking action. This occurred in December 2003, when the FDA announced a rule banning dietary supplements containing ephedrine alkaloids (**ephedra**), after reviewing the substantial number of adverse events (including death) associated with these products.

## DRUG PATENTS

A patent may confer legal protection of a drug's composition of matter, its use, or the process by which it is manufactured. **Composition of matter patents** grant exclusive rights to an individual chemical compound or a range of compounds, and may hint at synthesis or potential uses. **Use patents** grant exclusive rights to the uses that may result from research in a particular area and investigation in that area. **Process patents** grant exclusive rights to the complete process by which a compound is synthesized.

U.S. law grants a patent to a technology that is novel, useful, and is not obvious to someone with appropriate expertise. This technology may not be publicly disclosed for more than a year, even by those applying for patent. Application for a patent requires the applicant to disclose all work previously undertaken in that field, as well as the best anticipated use for the technology at the time. Patents in the United States are valid for a period of 17 years from the issue date if filed before June 1995 and for a period of 20 years from the filing date if filed after June 1995.

## *Conclusion and Future Directions*

In the United States, the federal FDA regulates all aspects of drug development and regulation. FDA approval of new drugs follows a lengthy process of preclinical and clinical studies. Each phase of study provides critical information that defines the study protocol for subsequent investigations. No collection of clinical trials can guarantee complete safety for all future patients. Thus, the FDA, in conjunction with drug manufacturers, continues to monitor the adverse effects, manufacturing processes, and overall safety of a drug for its lifetime. The FDA also regulates the marketing and sale of nonprescription medications and dietary supplements.

## *Suggested Readings*

Center for Drug Evaluation and Research, Food and Drug Administration, United States Department of Health and Human Services. The CDER Handbook. Revised 03/16/98. Available at http://www.fda.gov/cder/handbook/ (*Describes the processes by which the FDA evaluates and regulates drugs, including new drug evaluation and post-marketing monitoring of drug safety and effectiveness.*)

Goldstein I, Lue TF, Padma-Nathan H, et al. Oral sildenafil in the treatment of erectile dysfunction. N Engl J Med 1998;338:1397–1404. (*Randomized, controlled, double-blind phase III study of the efficacy and safety of sildenafil in the treatment of erectile dysfunction.*)

Nightingale, SL. Viagra approval information on the internet [from the Food and Drug Administration]. JAMA 1998;279:1684. (*Summarizes the basis for FDA approval of sildenafil, with link to Internet address containing FDA's review of the drug, approval letter, professional labeling, and information for consumers.*)

Salonia A, Rigatti P, Montorsi F. Sildenafil in erectile dysfunction: a critical review. Curr Med Res Opin 2003;19:241–262. (*Reviews the clinical literature on sildenafil, focusing on the results of long-term post-marketing studies of drug effectiveness and safety.*)

# 52

# Pharmacogenomics

Yeang Ch'ng, Ramy A. Arnaout, and Jeffrey M. Drazen

Introduction
Case
Physiology
    Genomic Variation in Human Populations
Pharmacology
    Variation in Target Proteins
    Variation in Enzymes of Drug Metabolism
    Idiosyncratic Effects
Conclusion and Future Directions
Suggested Readings

## INTRODUCTION

Previous chapters have described therapeutic medications in the context of their molecular targets. These targets are typically sufficiently common in the population as a whole—and sufficiently similar from person to person—that most medications act as intended in most people. There are, however, exceptions. Interindividual differences in the genes that encode these drug targets, or in genes that encode the proteins responsible for drug metabolism, can profoundly affect the success or failure of pharmacotherapy. The study of how these differences influence therapy is termed **pharmacogenomics** (or **pharmacogenetics**). This chapter introduces the field and describes how knowledge of interindividual differences in genomic makeup enables the individualization of optimal drug therapy.

## Case

It is May of 1975. Five interns at St. Mary's Hospital Medical School in England are taking part in a study of the effects of debrisoquin, an antihypertensive drug in use at the time. Blood pressure cuffs and containers for urine samples are arrayed nearby as each volunteer takes a single 40 mg pill. An hour passes without incident. Then one of the volunteers, a pharmacologist named Robert Smith, begins to feel dizzy and is unable to stand. Blood pressure measurements show severe orthostatic hypotension (70/50 mm Hg); Dr. Smith has to lie down to avoid syncope. Dr. Smith's symptoms gradually resolve, but he still has after-effects 2 days later. By contrast, his colleagues all feel fine the entire time. Once Dr. Smith feels better, he joins in his colleagues' intellectual interest in his disquieting experience.

### QUESTIONS

1. What happened to Dr. Smith? Why did he alone of the five volunteers experience symptoms?
2. Would other individuals be expected to react similarly? Would one out of five people be affected in other populations? Would women be more or less affected than men, or the elderly more or less affected than the young?
3. Should Dr. Smith worry that he might be predisposed to react adversely to other medications?

## PHYSIOLOGY

Three types of interindividual genetic variation affect pharmacotherapy: variations in target proteins or pathways, in enzymes of drug metabolism, and in idiosyncratic effects.

### GENOMIC VARIATION IN HUMAN POPULATIONS

The human genome contains about three billion nucleotides. Current best estimates are that the genome contains between 30,000 and 40,000 genes, which through alternative splicing and post-translational modification may code for 100,000 or more different proteins. Many of these proteins are involved in disease processes, and over the years, a number of them have also come to be used as targets for pharmacologic intervention.

The genomes of any two individuals—regardless of race, ethnicity, or geographic origin—are about 99.9% identical. This degree of similarity means that any two people differ on average at about one nucleotide in every thousand, for a total of three million differences between them. The majority of these differences are so-called **single nucleotide polymorphisms** or **SNPs** (pronounced "snips"), in which one nucleotide is exchanged for another at a given position. The remainder of the differences are insertions, deletions, duplications, and reshufflings, sometimes of just one or a few nucleotides, but occasionally of large parts of or even whole genes. Taken together, these differences constitute a person's genetic individuality. Some of this individuality includes how a particular person will respond to an administered drug.

SNPs and other differences can occur anywhere in the genome—on any chromosome, in any gene, or along any of the long stretches of noncoding sequences that separate one gene or gene fragment from another. To be important pharmacologically, differences generally must affect either the function or the amount of a protein. Hence, pharmacologically important DNA sequence differences tend to fall within genes: either within their coding sequences or in the promoters, enhancers, splice sites, or other sequences that control gene transcription and mRNA translation (Fig. 52-1). Current genomic technology allows several thousand polymorphisms to be screened for simultaneously.

With three million genetic differences separating any two persons, and a planet of over six billion people, one might expect that essentially every possible nonlethal mutation exists in the population many times over. As it happens, however, some genetic differences are much more common than others, presumably reflecting humanity's recent common ancestry and its history of genetic bottlenecks and population expansions. Studies of parts of genomes from people of diverse racial, ethnic, and geographic origin have shown that the vast majority of differences among human beings are accounted for by relatively few SNPs. Estimates from these studies are that only about 10 million SNPs are present at a frequency of 1% or higher in the overall population; the rest are far rarer. Of these 10 million "common" SNPs, only a few hundred thousand are likely to be found within coding sequences (so-called coding- or cSNPs), and of these, even fewer will result in amino acid changes, owing to the redundancy of the genetic code. Hence, most genome variation is unlikely to have major biologic implications, further reducing the number of differences likely to be important for tailoring pharmacotherapy. To date, about half of the 10 million common SNPs have been cataloged; however, most of the work of discovering what effect, if any, each difference has still lies ahead.

A related issue is how these differences are distributed throughout the population. Specifically, it is valuable clinically to know to what extent genetic variation correlates with membership in racial, ethnic, or geographic groups. In general, it is estimated that within-group differences account for between 93 and 97% of interindividual genetic variation, while between-group differences account for just 3 to 7%. However, within a given group, there are certain polymorphisms that many or most members share. For the most part, a person's geographic place of origin correlates well with his or her genetic background. Furthermore, there are large blocks of the genome in which recombination is rare. Each block tends to have its own signature SNPs, and members of a group tend to share blocks. A group's race, ethnicity, or geographic origin can, therefore, in principle be expected to help predict whether there will be substantial variance of response to a particular drug, even in absence of genetic data. The caveat, of course, is that individuals within any one group vary widely, so that group membership itself may provide little to no clinically useful information about a specific individual.

Overall, populations are characterized by relatively few biologically meaningful genetic differences: some will sort along recognizable geographic or other group lines; the majority are sprinkled across groups. Where present, these differences tend to be moderately common, generally occurring in at least 1% of the population. Where these differences have pharmacologic consequences, therefore, they are likely to affect a small but significant percentage of people. These are the people that individualization of pharmacotherapy will be most likely to help.

## PHARMACOLOGY

Genetic variations of three main types can modify responses to drugs. First, variation in target proteins, or more generally in target pathways, can make a medication that is effective for most people ineffective for some. Second, variation in enzymes that metabolize a drug can make a dose that is therapeutic for one person ineffective or toxic for another person;

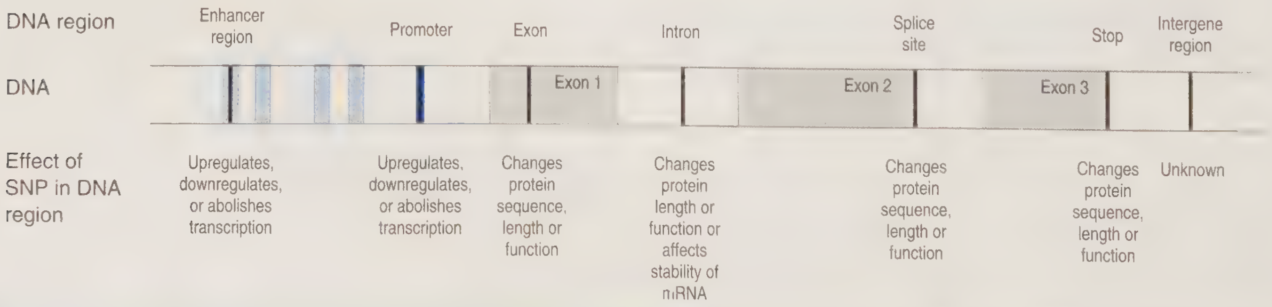

DNA region
Enhancer region
Promoter
Exon
Intron
Splice site
Stop
Intergene region
DNA
Exon 1
Exon 2
Exon 3
Effect of SNP in DNA region
Upregulates, downregulates, or abolishes transcription
Upregulates, downregulates, or abolishes transcription
Changes protein sequence, length or function
Changes protein length or function or affects stability of mRNA
Changes protein sequence, length or function
Changes protein sequence, length or function
Unknown

Figure 52-1. Some Possible Types and Locations for Genomic Variation. Most interindividual variation consists of single nucleotide polymorphisms (SNPs), denoted by black vertical lines. These and other variations can occur within coding regions (exons), or within noncoding regions such as enhancers, promoters, introns, splice sites, or the long stretches of DNA that separate one gene from another. The effect of the SNP depends in part on its location relative to the gene sequence, as noted.

variation in enzymes of metabolism can also lead to the formation of toxic metabolites in subsets of the population. Third, variation in genes unrelated to target proteins or enzymes of metabolism can result in idiosyncratic (and typically unpredictable) responses or dangerous side effects. The remainder of this chapter discusses these three types of variation in more detail, and provides examples.

## VARIATION IN TARGET PROTEINS

Pharmacologic agents generally exert their effect by interacting with particular target proteins. These target proteins are almost always involved in the biochemical pathways of the disease processes that the drugs are being used to treat. Variation in target proteins or their pathways can, therefore, strongly influence the outcome of pharmacotherapy (Table 52-1). Recall that variation may change the amount of protein that is produced, as well as alter a protein's structure and function. As a result, variation that affects regulatory sequences such as promoters, enhancers, and splice sites can be as important pharmacologically as variation that affects amino acid sequences in the regions that determine the protein's identity.

Examples of both kinds of variation have been described in the pharmacogenomics of asthma. As noted in Chapter 45, Integrative Inflammation Pharmacology: Asthma, the recently withdrawn anti-asthma medication **zileuton** decreases airway inflammation by inhibiting the enzyme **5-lipoxygenase.** This enzyme is encoded by a gene called *ALOX5*. This gene displays polymorphism in its promoter region, specifically in the number of tandem repeats of the sequence GGGCGG. These repeats bind the transcription factor complex Sp1, which upregulates transcription of *ALOX5*. The most common allelic polymorphism contains five repeats and is present in about 77% of the alleles in the general population. The next most common polymorphisms contain four and three repeats, present at about 17% and 4%, respectively. These polymorphisms are in Hardy–Weinberg equilibrium in the population, and so about 94% of the population has at least one copy of the five-repeat allele. Because of increased Sp1 binding, people who have the five-repeat allele are thought to produce more 5-lipoxygenase than people who lack it. Interestingly, there seems to be no relationship between the presence or absence of the five-repeat allele and the severity of asthma in the population; in other words, polymorphism in the *ALOX5* promoter seems not to affect the disease process itself, at least not monogenically. However, in trials of a 5-lipoxygenase inhibitor related to zileuton, only subjects who had one copy of the five-repeat allele showed any response (measured according to the standard of at least a 12% improvement in forced expiratory volume in one second [$FEV_1$] by the end of the trial period). This result suggests that zileuton-like compounds are unlikely to help the 6% of the population that lacks the five-repeat allele; identifying them allows the use of alternative medications that would be more effective. This example also illustrates an important principle of pharmacogenomics: a polymorphism need not cause a disease to influence its treatment.

The second example from asthma pharmacotherapy involves the gene for the **$\beta_2$ adrenergic receptor.** Recall from Chapter 8, Adrenergic Pharmacology, that the $\beta_2$ adrenergic receptor mediates the relaxation of smooth muscle in small airways, decreasing the asthmatic obstruction to air flow. Thus, adrenergic agonists—a class of drugs that includes **albuterol,** which is commonly used in inhalers—are often prescribed for use in the treatment of asthma. The $\beta_2$ adrenergic receptor is highly polymorphic, and most attention has been focused at amino acid position 16. At this position, approximately 60% of people of Hispanic or Caucasian descent have a cSNP that encodes glycine (G16), whereas the other 40% of the population has a cSNP encoding arginine (R16): individuals with the G16 variant acutely downregulate the receptor in response to β-agonist use. This response makes β-agonists less effective in individuals that have the G16 allele than in individuals who lack it. In a study of children comparing differences in $FEV_1$ before and after albuterol administration, G16 homozygotes were 50% less likely to respond to albuterol than R16 homozygotes and 25% less likely to respond than R16-G16 heterozygotes. Again, this result was independent of the severity or even the presence of disease. The finding that G16 homozygotes are less likely to respond to albuterol is a first step toward developing an effective pharmacologic strategy for such patients.

Because demonstrating how polymorphisms affect drug efficacy generally requires large, well-controlled trials—and because the relationships among polymorphism, medication, and disease are often complex—the precise pharmacologic effect of a given polymorphism is often difficult to elucidate. An example from the pharmacogenomics of cholesterol-lowering therapy illustrates this difficulty.

As noted in Chapter 22, Pharmacology of Cholesterol and Lipoprotein Metabolism, the statins are a family of drugs that lower cholesterol by inhibiting sterol synthesis. They act by inhibiting the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in cholesterol synthesis. This inhibition also results in upregulation of low-density lipoprotein (LDL)

**TABLE 52-1** Illustrative Polymorphisms in Drug Targets

| PROTEIN | AFFECTED DRUG CLASS (EXAMPLE) |
|---|---|
| $\beta_2$-adrenergic receptor | β-adrenergic agonists (albuterol) |
| 5-lipoxygenase | Zileuton |
| Angiotensin converting enzyme | ACE inhibitors (lisinopril) |
| Apolipoprotein E | Statins (pravastatin) |
| Sulfonylurea receptor | Tolbutamide |
| 5-hydroxytryptamine receptors | Neuroleptics (clozapine) |

receptors, which leads to increased clearance of LDL, thereby lowering blood cholesterol. In turn, lower blood cholesterol helps to minimize atherosclerosis. Statins are very effective cholesterol-lowering medications: understanding how genetic polymorphisms may temper the effectiveness of statins in some individuals has been a major research effort.

Consider the polymorphism in **ApoE,** an apolipoprotein involved in clearance of cholesterol from the blood, encoded by the apoE gene. This gene has three common alleles, called ε2, ε3, and ε4, which are found at frequencies of 8%, 78%, and 14%, respectively, in the general population. These alleles differ from each other at sites that correspond to two amino acid positions: ε2 codes for cysteines at both positions; ε3, a cysteine and an arginine; and ε4, two arginines. These differences influence how well the protein binds to lipoprotein receptors, and consequently how well these receptors clear cholesterol: the ε4 ApoE binds most effectively; ε3, second best; and ε2, least well. Paradoxically, the allele that carries the greatest risk of high cholesterol and atherosclerosis is ε4: this is because rapid clearance results in a downregulation of LDL receptors and a secondary rise in LDL in the blood. Importantly, HMG-CoA reductase activity is also downregulated. Because of this downregulation, HMG-CoA reductase activity is constitutively low, so that statins might not be expected to be as beneficial for the 2% of the population that is homozygous for ε4 as for heterozygotes who carry ε2 or ε3. Indeed, a number of studies have borne out this expectation. Additional studies have suggested that the pharmacogenomic effect is limited to men. Still another study of heart attack survivors found that, although patients who had the ε4 allele were twice as likely to die in 5 years as patients who did not have the allele, statin therapy removed this risk entirely, and resulted in lower 5-year mortality for patients with the ε4 allele than for those without it.

ApoE is one of a dozen or more proteins in which polymorphisms are thought to effect the efficacy of statins. Clearly, more investigation is required.

## VARIATION IN ENZYMES OF DRUG METABOLISM

Genetic variation in the enzymes of drug metabolism is perhaps the most important factor responsible for interindividual variation with respect to responses to medications. Indeed, recognition of metabolic variation is what first led to the development of pharmacogenetics in the 1950s. The enzymes of metabolism were introduced in Chapter 4, Drug Metabolism. There are two broad categories of metabolic reactions: phase I reactions, which add hydroxyls, thiols, or other groups to inactivate compounds, and phase II reactions, which add groups such as glucuronic acid to enhance solubility of compounds for excretion. Phase I and phase II reactions do not necessarily occur in that order, and metabolic intermediates may be pharmacologically active. Indeed, some medications are administered as inactive prodrugs that must undergo phase I or phase II reactions before they can exert their pharmacologic activity.

Polymorphism is common in the enzymes of drug metabolism: clinically significant polymorphisms have been found in nearly all of the major enzymes involved in both phase I and phase II reactions (Table 52-2). Interestingly, most of these polymorphisms produce no apparent clinical effects in the absence of drug therapy, because many metabolic enzymes lack critical endogenous substrates, and no completely inactivating polymorphisms have been found in phase I enzymes that do have critical endogenous substrates. There may also be functional redundancy for endogenous compounds among enzymes; however, there is no guarantee that redundancy will exist for the metabolism of exogenous substrates, such as medications. Hence, enzyme polymorphisms can result in clinically significant differences in efficacy and toxicity of medications from one person to another. This presents an important challenge and opportunity for the individualization of pharmacotherapy.

Several factors influence the likelihood that a given polymorphism will affect drug therapy. Important polymorphisms are typically those involved either in major metabolic pathways or in minor pathways that generate potent toxic metabolites. The therapeutic index also plays a role: small variations in metabolism of drugs with low therapeutic indices may yield clinically significant concentration of toxic metabolites. Moreover, variations in receptor densities or sensitivities can interact with metabolic variations, further complicating drug efficacy at the individual level.

Consider two classic examples that illustrate how variation in the enzymes of drug metabolism can affect drug choice, dose, and outcome of pharmacotherapy. The first involves the antimycobacterial agent **isoniazid** (see Chapter 35, Pharmacology of the Bacterial Cell Wall), which is metabolized through acetylation by a phase II enzyme. Observations of differences among patients in blood levels of this drug were made shortly after its introduction, in the 1950s. It was soon found that these differences were caused by differences in rates of acetylation; patients were found to be either "slow acetylators," who metabolized the drug slowly, or "fast acetylators," who metabolized isoniazid quickly (Fig. 52-2). Pedigree studies showed that acetylator phenotype was genetically controlled. Slow acetylators were found to be homozygous for a particular allele, whereas fast acetylators were found to be homozygous or heterozygous for a different allele of the same protein, which is now known to belong to the **N-acetyltransferase** (NAT) family of phase II liver enzymes. NAT enzymes were later found to be involved in the metabolism of a number of other drugs and xenobiotics. Indeed, the slow-acetylator phenotype is thought to be related to a number of toxicities that result from accumulation of supratherapeutic drug levels, including sulfonamide hypersensitivity, hydralazine- and procainamide-induced lupus, and isoniazid-induced neurotoxicity.

NAT polymorphisms may also account for interindividual differences in susceptibilities to chemical carcinogenesis. For example, NAT2 (see below) slow acetylators are at increased risk for developing bladder cancer, apparently because various carcinogenic aromatic amines (such as in tobacco smoke) are less effectively detoxified in such individuals. On the other hand, NAT2 fast acetylators may be at increased risk of heterocyclic amine-induced colon cancer because O-acetylation activates these heterocycles, making metabolites the proximate carcinogens.

Most of the polymorphism in drug acetylation can be ascribed to variation in the *NAT2* gene, in which there are seven common cSNPs that lead to amino acid changes. Some of these polymorphisms (G191A, C282T, and T341C) result in the slow-acetylator phenotype, when present in two copies. A different polymorphism (A803G) leads to the fast-acetylator phenotype, as does the most common *NAT2* polymorphism.

**TABLE 52-2** Illustrative Polymorphisms in Drug Metabolism

| ENZYME | AFFECTED MEDICATION, CLASS, OR COMPOUND |
|---|---|
| **↑ Phase I Enzymes** | |
| CYP1A2 | Acetaminophen, caffeine, propranolol |
| CYP1B1 | Estrogen metabolites |
| CYP2A6 | Halothane, nicotine |
| CYP2B6 | Aflatoxin, cyclophosphamide, mephenytoin |
| CYP2C8 | Paclitaxel, retinoic acid |
| CYP2C9 | Nonsteroidal anti-inflammatory drugs, phenytoin, warfarin |
| CYP2C19 | Omeprazole, phenytoin, propranolol |
| CYP2D6 | Antidepressants, antipsychotics, β-adrenergic blockers, codeine, dextromethorphan, flecanide, methoxyamphetamine, phenformin, propafenone |
| CYP2E1 | Acetaminophen, ethanol |
| CYP3A5 | Calcium channel blockers, cyclosporine, dapsone, etoposide, lidocaine, lovastatin, macrolides, midazolam, quinidine, steroids, tacrolimus, tamoxifen |
| Aldehyde dehydrogenase | Cyclophosphamide, ethanol, vinyl chloride |
| **↑ Phase II Enzymes** | |
| N-acetyltransferase 1 | Aspirin, sulfamethoxazole |
| N-acetyltransferase 2 | Caffeine, dapsone, hydralazine, isoniazid, procainamide, sulfonamides |
| Glutathione S-transferase | Ethacrynic acid, retinoic acid |
| Sulfotransferase steroids | Acetaminophen, dopamine, epinephrine, estrogens, |
| Catechol-*O*-methyltransferase | Ascorbic acid, estrogen, levodopa |
| Histamine methyltransferase | Histamine |
| Thiopurine methyltransferase | Azathioprine, mercaptopurine, thioguanine |
| UDP-glucuronosyltransferase | Androgens, ibuprofen, irinotecan, naproxen, morphine |

Note that for enzymes of drug metabolism, a more active polymorphism will usually compensate for the decreased activity of a less active polymorphism, such that the slow-acetylator phenotype is recessive.

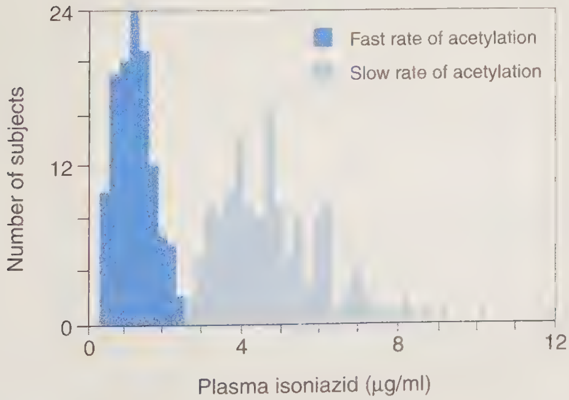


**Figure 52-2. Example of Pharmacogenetics.** Plasma isoniazid concentrations were measured in 267 subjects 6 hours after an oral dose of the compound. The bimodal distribution in the rate of acetylation is attributable to genetic polymorphisms within the N-acetyltransferase 2 gene. If a plasma isoniazid value of 2.5 μg/ml is used as the cutoff, this bimodal distribution can be used to categorize patients as "fast acetylators" or "slow acetylators."

The second example of variation in enzymes of drug metabolism involves a polymorphism that affects various medications used to treat depression and arrhythmias. This polymorphism was discovered in the 1970s, when investigators noticed that there were significant variations among individuals in the oxidative metabolism of **debrisoquin,** an early sympatholytic antihypertensive, and of **sparteine,** an alkaloid that was then used as an antiarrhythmic. The role of Dr. Robert L. Smith, an English pharmacologist, in this discovery was recounted in the introductory case. Variation in the metabolism of both drugs was found to result from polymorphism in a single gene. So-called "poor metabolizers," such as Dr. Smith, were homozygous for a recessive allele of this gene; "extensive metabolizers," who comprised the rest of the population, were heterozygous or homozygous for a different allele. Since this discovery, a rarer subset of "ultrarapid metabolizers" has also been found: these individuals carry a duplicate copy of the gene (Fig. 52-3). In the 1980s, the gene was isolated and found to be *CYP2D6,* a member of the cytochrome P450 family of phase I enzymes. The poor-metabolizer phenotype is common in many populations. It has been estimated that 5–10% of Caucasians are poor metabolizers; hence the likelihood is relatively high that other people in England might have side effects similar to those Dr. Smith experienced. In Asians, in contrast, the poor-metabolizer phenotype is present at just 1–2%. The ultrarapid metabolizer phenotype, rare in most Caucasian populations, is found in 3% of Spaniards and 13% of Ethiopians. These differences have important medical implications, because

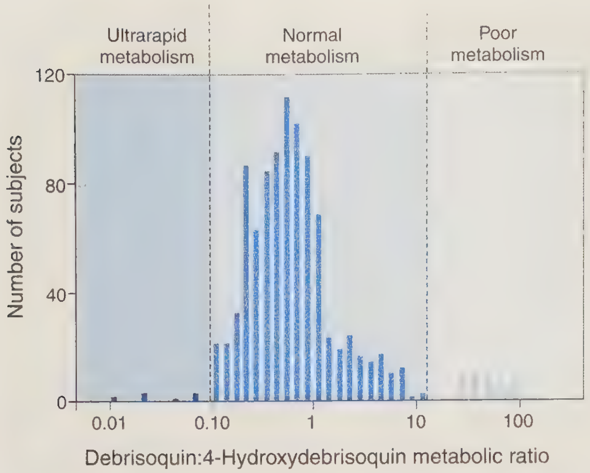


**Figure 52-3. Pharmacogenetics of CYP2D6.** Urinary metabolic ratios of debrisoquin to its metabolite 4-hydroxydebrisoquin are shown for 1,011 Swedish subjects. Most subjects had ratios between 0.10 and 10; these subjects metabolize debrisoquin extensively. Subjects with a ratio less than 0.10 have ultrarapid metabolism of debrisoquin, while those with a ratio above 10 have poor metabolism. Debrisoquin is metabolized by CYP2D6, and differences in metabolism reflect genetic polymorphisms in this enzyme. Because CYP2D6 metabolizes many drugs, such polymorphisms can have significant implications for drug dosing and toxicity.

CYP2D6 metabolizes many commonly prescribed medications (Table 52-2). These drugs include the β-adrenergic antagonist metoprolol, the antiarrhythmic flecainide, the neuroleptic haloperidol, the opiods codeine and dextromethorphan, and the antidepressants fluoxetine, imipramine, and desipramine, among many others. CYP2D6 is also involved in the metabolism of amphetamines such as Ecstasy. Hence, Dr. Smith might well be concerned about experiencing side effects to numerous other medications. For poor metabolizers, dosing of medications metabolized by CYP2D6 should be decreased to avoid the risk of toxicity. On the other hand, ultrarapid metabolizers require unusually high doses of these same medications to achieve a therapeutic effect. An individual's phenotype in this regard can be determined through the urinary metabolic ratio (UMR) test, which involves measuring urinary excretion of a specific metabolite after administration of a test drug.

## IDIOSYNCRATIC EFFECTS

The third way in which genetic variation influences pharmacotherapy is in idiosyncratic effects (Table 52-3). These effects differ from the previous examples in that they are not known to be caused by differences in target proteins or pathways or differences in enzyme metabolism. Instead, idiosyncratic effects seem to result from chance interactions between the medication and some unusual aspect of the physiology of the individual. Two classic examples illustrate this least predictable effect of interindividual genetic variation.

Factor V Leiden (discovered in Leiden, Netherlands) is a polymorphism in a clotting factor that predisposes to thrombosis (see Chapter 21, Pharmacology of Hemostasis and Thrombosis). This polymorphism is caused by a cSNP that results in the substitution of a glutamine for an arginine at amino acid position 506 (R506Q). This substitution makes factor V resistant to cleavage and thus potentiates its physiologic prothrombotic effect. Heterozygote carriers and homozygotes are, respectively, 7 and 80 times more likely to develop a deep venous thrombosis than those who do not carry the Leiden polymorphism. The polymorphism is remarkably widespread: it is estimated that some 3% of the world's population carries it, including some 6 to 8% of Northern Europeans. The Leiden polymorphism is estimated to account for one quarter of all patients who present with a history of repeated deep venous thromboses and pulmonary emboli. A noncoding SNP in the prothrombin gene, G20210A, results in increased transcription and, therefore, also predisposes to thrombosis. Women with polymorphisms that predispose to thrombosis are more likely to develop thromboses while taking oral contraceptive pills (OCPs). This idiosyncratic effect does not result from the OCP's planned purpose of preventing pregnancy, nor is it related to drug metabolism. Instead, it is an indirect result of the hypercoagulable state, induced by estrogens, through increased hepatic synthesis of clotting factors.

A second example of idiosyncratic effects involves functional deficiency of the enzyme glucose 6-phosphate dehydrogenase (G6PD; see Chapter 37, Pharmacology of Parasitic Infections). This enzyme is involved in protecting red blood cells from oxidative injury. Although G6PD deficiency is generally considered a single entity, there are a number of polymorphisms that lead to this condition. The most common one involves a cSNP that causes an amino acid substitution resulting in a reduction of enzyme function by 90%–95%. This allele, called A$^-$, is present in 10%–20% of African Americans,

**TABLE 52-3** Illustrative Polymorphisms Resulting in Idiosyncratic Side Effects

| PROTEIN | SIDE EFFECT | ASSOCIATED AGENTS |
|---|---|---|
| Factor V Leiden | Deep vein thrombosis | Oral contraceptives, smoking |
| Glucose 6-phosphate dehydrogenase | Hemolysis if deficient | Isoniazid, sulfonamides, chloramphenicol, primaquine, ibuprofen, nitrofurantoin |
| Ion channels | Long QT interval | Erythromycin, clarithromycin, quinidine |
| Apolipoprotein E | ε4 allele linked to poorer response than ε3 to tacrine in Alzheimer's disease | Tacrine |

and is thought to provide some protection against malaria. A different inactivating polymorphism is found at lesser frequencies among people of Mediterranean, Middle Eastern, Indian, and South-East Asian descent; a third is also present in South-East Asia. A number of medications put oxidative stress on red blood cells as a side effect unrelated to their intended targets or their clearance via metabolism. These medications include sulfonamides, antimalarials, and the analgesics acetaminophen and ibuprofen. Individuals with G6PD deficiency who are exposed to these medications may develop acute and sometimes severe hemolytic anemia.

By definition, idiosyncratic effects are difficult to predict. Structural information emerging from genomic and proteomic investigations may prove useful in pharmacogenomic screens for unexpected drug interactions in the future, but for now predicting idiosyncratic effects remains elusive.

## Conclusion and Future Directions

Pharmacogenomics is the study of how variations in people's genes affect how they respond to medications: the goal is to maximize effectiveness and minimize toxicity based on an individual's genetic make-up. Genetic variation in target proteins or pathways, enzymes of drug metabolism, or unrelated proteins can lead to diminished effectiveness or increased toxicity. Identifying crucial genetic differences allows prescribers to choose different medications, or to titrate doses to avoid unwanted outcomes. Genomics technology is making it possible to scan individuals' genomes for a limited, predetermined set of polymorphisms known to affect certain forms of pharmacotherapy.

## Suggested Readings

### General Principles

Broder S, Venter JC. Sequencing the entire genomes of free-living organisms: the foundation of pharmacology in the new millennium. Ann Rev Pharmacol Toxicol 2000;40:97–132. (*Overview on genome sequencing and primer on the implications of genetic diversity for pharmacology.*)

Evans WE, McLeod HL. Pharmacogenetics—drug disposition, drug targets, and side effects. N Engl J Med 2003;348:538. (*Excellent review, with more emphasis on genomics.*)

Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999;286:487–491. (*Overview of the major kinds of pharmacogenomic variation, especially in enzymes of metabolism, with numerous supplementary examples available online.*)

Weinshilboum R. Inheritance and drug response. N Engl J Med 2003;348:529. (*Review of how genetic and genomic factors affect drug response.*)

### The Case of Dr. Smith

Smith RL. The Paton Prize Award. The discovery of the debrisoquin hydroxylation polymorphism: scientific and clinical impact and consequences. Toxicology 2001;168:11–19. (*First-person account of the discovery of the slow metabolizer phenotype, including the author's own role.*)

### Genomic Variation

Gabriel SB, Schaffner SF, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–2390. (*Describes the existence of blocks of genetic sequences that correlate with geographic background.*)

Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234–236. (*Discussion of variation in human populations, with a focus on SNPs.*)

Rosenberg NA, Pritchard JK, et al. Genetic structure of human populations. Science 2002;298:2381–2386. (*This study showed that, despite the vastly greater amount of genetic difference within populations than between them, common genetic differences do correlate with geographic and population origins.*)

### Specific Topics

Drazen JM, Yandara CN, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Med 1999;22:168–171. (*Original study that showed different pharmacological responses in people with different polymorphisms of the ALOX5 gene.*)

Maitland-van der Zee A.-H, Klungel OH, et al. Genetic polymorphisms: importance for response to HMG CoA reductase inhibitors. Atherosclerosis 2002;163:213–222. (*Review of the various polymorphisms currently thought to influence the effectiveness of statins in the general population.*)

Online Mendelian Inheritance in Man, OMIM (TM). McKusick–Nathans Institute for Genetic Medicine, The Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000. www.ncbi.nlm.nih.gov/omim/ (*Resource for learning more about particular genes and polymorphisms.*)

# CREDIT LIST

Figure 1-1: Adapted from an illustration (www.genome.gov/Pages/Hyperion//DIR/VIP/Glossary/Illustration/protein.shtml) on the National Human Genome Research Institute web site: www.nhgri.nih.gov

Figure 1-2: Data used to render the image in panel A were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1FPU) by Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J (Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. Science 2000;289:1938–1942, Figure 1). Panels B and C were adapted with permission from Schindler T, et al. (ibid., Figures 1 and 2).

Figure 2-7: Adapted with permission from Stephenson RP. A modification of receptor theory. Brit J Pharmacol 1956;11:379–393, Figure 10.

Figure 3-1: Adapted with permission from Hardman JG, Limbird LE, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th Ed, Figure 1-1, page 3. New York: The McGraw-Hill Companies, 2001.

Figure 3-7: Adapted with permission from Katzung BG, ed. Basic & Clinical Pharmacology, 7th Ed, Figure 3-2, page 38. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998.

Figure 4-2A: Adapted with permission from Katzung BG, ed. Basic & Clinical Pharmacology, 7th Ed, Figure 4-3, page 52. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998.

Figure 6-14: Adapted with permission from Goldstein GW, Laterra J. Appendix B: Ventricular organization of cerebrospinal fluid: blood-brain barrier, brain edema, and hydrocephalus. In: Kandel ER, Schwartz JH, Jessell TM, eds. Principles of Neural Science, 4th Ed, Figure B-4, page 1291. New York: The McGraw-Hill Companies, 2000.

Figure 7-2: Adapted with permission from Changeux JP. Chemical signaling in the brain. Sci Am 1993;269:58–62.

Figure 7-4: Adapted with permission from Kandel ER, Schwartz JH, Jessell TM, eds. Principles of Neural Science, 4th Ed, Figure 11-1, page 188. New York: The McGraw-Hill Companies, 2000.

Table 7-4: Adapted with permission from Hardman JG, Limbird LE, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th Ed, Table 7-1, page 159. New York: The McGraw-Hill Companies, 2001.

Table 8-1: Adapted with permission from Hardman JG, Limbird LE, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th Ed, Table 6-3, page 137. New York: The McGraw-Hill Companies, 2001.

Figure 9-2: Adapted with permission from LaMotte RH, Campbell JN. Comparison of responses of warm nociceptive C-fiber afferents in monkey with human judgments of thermal pain. J Neurophysiol 1978;41:509-528 and Gybels J, Handwerker HO, Van Hees J. A comparison between the discharges of human nociceptive nerve fibers and the subject's ratings of his sensations. J Physiol (Lond) 1979;292:193–206.

Figure 9-10: Adapted with permission from Smith C. Pharmacology of local anesthetic agents. Brit J Hosp Med 1994;52:455–460.

Table 9-1: Adapted with permission from Carpenter RL, Mackey DC. Local anesthetics. In: Barash PG, Cullen BF, Stoelting RK, eds. Clinical Anesthesia, 2nd Ed, pages 509–541. Philadelphia: Lippincott, 1992.

Figure 10-2B: Adapted with permission from Cooper JR, Bloom FE, Roth RN. Biochemical Basis of Neuropharmacology, 7th Ed, Figures 6-1 and 6-11. New York: Oxford University Press, 1996.

Figure 10-10: Adapted with permission from Neelands TR, Greenfield J, Zhang J, Turner RS, Macdonald RL. $GABA_A$ receptor pharmacology and subtype mRNA expression in human neuronal NT2-N cells. J Neurosci 1998;18:4993–5007, Figure 1a.

Figure 10-11: Adapted with permission from Neelands TR, Greenfield J, Zhang J, Turner RS, Macdonald RL. $GABA_A$ receptor pharmacology and subtype mRNA expression in human neuronal NT2-N cells. J Neurosci 1998;18:4993–5007, Figure 6c.

Figure 10-12: Adapted with permission from O'Shea SM, Wong LC, Harrison NL. Propofol increases agonist efficacy at the $GABA_A$ receptor. Brain Res 2000;852:344–348, Figure 4B.

Figure 11-4: Adapted with permission from Hardman JG, Limbird LE, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th Ed, Figure 22-5, page 554. New York: The McGraw-Hill Companies, 2001.

Figure 11-5: Adapted with permission from Seeman P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptor, clozapine occupies D4. Neuropsychopharmacology 1992;7:261–284, Figure 2.

Figure 11-10: Adapted with permission from Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987;1:133–152.

Figure 13-3: Adapted with permission from Lothman EW. Pathophysiology of seizures and epilepsy in the mature and immature brain: Cells, synapses and circuits. In: Dodson WE, Pellock JM, eds. Pediatric Epilepsy: Diagnosis and Therapy, pages 1-15. New York: Demos Publications, 1993.

Figure 13-4: Adapted with permission from Lothman EW. The neurobiology of epileptiform discharges. Am J EEG Technol 1993;33:93–112.

Figure 13-5A: Adapted with permission from Kandel ER, Schwartz JH, Jessell TM, eds. Principles of Neural Science, 4th Ed, Figure 45-9, page 899. New York: The McGraw-Hill Companies, 2000.

Figure 14-2: Adapted from Miller KW. General anesthetics. In: Wolff ME, ed. Burger's Medicinal Chemistry and Drug Discovery, Volume 3: Therapeutic Agents, 5th Ed, Figure 36-2, 1996. This material used by permission of John Wiley & Sons, Inc.

Figure 14-6: Adapted with permission from Eger EI. Anesthetic Uptake and Action, Figure 4-7. Baltimore: Williams & Wilkins, 1974.

Figure 14-7: Adapted from Eger EI. Uptake and distribution. In: Miller RD, ed. Anesthesia, 5th Ed, Figure 4-2. Philadelphia: Churchill Livingstone, 2000. With permission from Elsevier.

Figure 14-9: Adapted with permission from Eger EI. Anesthetic Uptake and Action, Figure 7-1 and Figure 7-8. Baltimore: Williams & Wilkins, 1974.

Figure 14-10: Adapted from Eger EI. Uptake and distribution. In: Miller RD, ed. Anesthesia, 5th Ed, Figure 4-10. Philadelphia: Churchill Livingstone, 2000. With permission from Elsevier.

Figure 14-12: Adapted with permission from Eger EI. Anesthetic Uptake and Action, Figure 14-8. Baltimore: Williams & Wilkins, 1974.

Figure 14-13: Adapted with permission from Trevor AJ, Miller RD. General anesthetics. In: Katzung BJ, ed. Basic & Clinical Pharmacology, 7th Ed, Figure 25-6, page 421. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998.

Figure 16-5: Adapted from Jones RT. The pharmacology of cocaine smoking in humans. In: Chiang CN, Hawks RL, eds. NIDA Research Monograph 99 (Research Findings on Smoking of Abused Substances), pages 30-41, 1990.

Figure 17-1: Adapted from Ackerman M, Clapham DE. Normal cardiac electrophysiology. In: Chien KR, Breslow JL, Leiden JM, Rosenberg RD, Seidman CE, eds. Molecular Basis of Cardiovascular Disease: A Companion to Braunwald's Heart Disease, Figure 12-1, page 282. Philadelphia: WB Saunders, 1999. With permission from Elsevier.

Figure 17-2: Adapted from Ackerman M, Clapham DE. Normal cardiac electrophysiology. In: Chien KR, Breslow JL, Leiden JM, Rosenberg RD, Seidman CE, eds. Molecular Basis of Cardiovascular Disease: A Companion to Braunwald's Heart Disease, Figure 12-2, page 284. Philadelphia: WB Saunders, 1999. With permission from Elsevier.

Figure 17-3: Adapted from Ackerman M, Clapham DE. Normal cardiac electrophysiology. In: Chien KR, Breslow JL, Leiden JM, Rosenberg RD, Seidman CE, eds. Molecular Basis of Cardiovascular Disease: A Companion to Braunwald's Heart Disease, Figure 12-1 and Figure 12-2, pages 282 and 284. Philadelphia: WB Saunders, 1999. With permission from Elsevier.

Figure 17-5: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 11.7, page 241. Baltimore: Williams & Wilkins, 1998.

Figure 17-6: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 11.8, page 241. Baltimore: Williams & Wilkins, 1998.

Figure 17-7: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 11.9, page 243. Baltimore: Williams & Wilkins, 1998.

Figure 17-9A: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.11B, page 371. Baltimore: Williams & Wilkins, 1998.

Figure 17-10: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.11A, page 371. Baltimore: Williams & Wilkins, 1998.

Figure 17-11: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.12, page 376. Baltimore: Williams & Wilkins, 1998.

Figure 17-12: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.13, page 377. Baltimore: Williams & Wilkins, 1998.

Figure 17-13: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.14, page 380. Baltimore: Williams & Wilkins, 1998.

Figure 18-1: Adapted with permission from Katz AM. Congestive heart failure: role of altered myocardial cellular control. N Engl J Med 1975;293:1184–1191 and Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 1.9, page 11. Baltimore: Williams & Wilkins, 1998.

Figure 18-2: Adapted with permission from Katz AM. Physiology of the Heart, 2nd Ed, Figure 8.4, page 187. New York: Raven Press, 1992.

Figure 19-10: Adapted from Skorecki KL, Brenner BM. Body fluid homeostasis in congestive heart failure and cirrhosis with ascites. Am J Med 1982;72:323–338, Figure 1. With permission from Elsevier.

Figure 19-11: Adapted with permission from Seldin DW, Giebisch G, eds. The Kidney: Physiology and Pathophysiology, 3rd Ed, Figure 54-8, page 1494. Philadelphia: Lippincott Williams & Wilkins, 2000.

Figure 19-12: Adapted with permission from Katzung BG, ed. Basic & Clinical Pharmacology, 8th Ed, Figure 11-6, page 173. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 2001.

Figure 20-1: Adapted with permission from Greineder K, Strichartz GR, Lilly LS. Basic cardiac structure and function. In: Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 1.7, page 9. Baltimore: Williams & Wilkins, 1998. Adapted from Berne RM, Levy MN. Control of cardiac output: coupling of heart and blood vessels. In: Cardiovascular Physiology, Figure 9.2. St. Louis: Mosby Year Book, 1997. With permission from Elsevier.

Figure 20-10: Adapted with permission from Benowitz NL. Antihypertensive agents. In: Katzung BG, ed. Basic & Clinical Pharmacology, 7th Ed, page 168. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998 and Kalkanis S, Sloane D, Strichartz GR, Lilly LS. Cardiovascular drugs. In: Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 17.7, page 360. Baltimore: Williams & Wilkins, 1998.

Figure 21-1: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 5-5. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 21-2: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 5-7. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 21-3: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 5-7. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 21-11: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 5-12. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 22-1: Adapted from Larsen PR, Kronenberg HM, et al., eds. Williams Textbook of Endocrinology, 10th Ed, Figure 34-5. Philadelphia: WB Saunders, 2003. With permission from Elsevier.

Figure 22-5: Adapted with permission from Quinn MT, Parthsarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci USA 1987;84:2995–2998, Figure 1.

Figure 22-7: Adapted from Vaughan CJ, Gotto AM Jr, Basson CT. The evolving role of statins in the management of atherosclerosis. J Am Coll Cardiol 2000;35:1–10. With permission from Elsevier.

Figure 23-1: Adapted with permission from Deshmukh R, Smith A, Lilly LS. Hypertension. In: Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 13.3, page 270. Baltimore: Williams & Wilkins, 1998.

Figure 23-2: Adapted with permission from Deshmukh R, Smith A, Lilly LS. Hypertension. In: Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 13.10, page 286. Baltimore: Williams & Wilkins, 1998.

Figure 23-5: Adapted with permission from Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 6.5, page 141. Baltimore: Williams & Wilkins, 1998.

Figure 23-6: Adapted from Gould KL, Lipscomb K. Effects of coronary stenoses on coronary flow reserve and resistance. Am J Cardiol 1974;34:48–55, Figure 2. With permission from Elsevier.

Figure 23-8: Adapted with permission from Frankel SK, Fifer MA. Heart failure. In: Lilly LS, ed. Pathophysiology of Heart Disease, 2nd Ed, Figure 9.5, page 199. Baltimore: Williams & Wilkins, 1998.

Figure 23-9: Adapted with permission from Harvey RA, Champe PC, eds. Lippincott's Illustrated Reviews: Pharmacology, Figure 16-6, page 157. Philadelphia: Lippincott Williams & Wilkins, 1992.

Table 23-1: Data (available at hin.nhlbi.nih.gov/nhbpep_slds/jnc/jncp2_2.htm) from The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, 2003 (available on the National Heart, Lung, and Blood Institute web site at www.nhlbi.nih.gov/guidelines/hypertension/).

Figure 26-2: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 26-27. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 26-8: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 26-27. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 27-5: Adapted with permission from Thorneycroft IH, Mishell DR Jr, Stone SC, Kharma KM, Nakamura RM. The relation of serum 17-hydroxyprogesterone and estradiol-17β levels during the human menstrual cycle. Am J Obstet Gynecol 1971;111:947–951.

Figure 28-4: Adapted with permission from Braunwald E, Fauci AS, et al., eds. Harrison's Principles of Internal Medicine, 15th Ed, Figure 333-4. New York: The McGraw-Hill Companies, 2001.

Figure 30-5: Adapted from Haskell CM, ed. Cancer Treatment, 3rd Ed, Figure 1.2, page 5. Philadelphia: WB Saunders Company, 1990. With permission from Elsevier.

Figure 32-2C: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1AFZ) by Zegar IS, Stone MP (Solution structure of an oligodeoxynucleotide containing the human N-Ras codon 12 sequence refined from 1H NMR using molecular dynamics restrained by nuclear overhauser effects. Chem Res Toxicol 1996;9:114–125).

Figure 32-4: Adapted with permission from Dekker NH, Rybenkov VV, et al. The mechanism of type IA topoisomerases. Proc Natl Acad Sci USA 2002;99:12126–12131, Figure 1.

Figure 32-5: Adapted with permission from Berger JM, Gamblin SJ, Harrison SC, Wang JC. Structure and mechanism of DNA topoisomerase II. Nature 1996;379:225–232, Figure 5.

Figure 32-6: Adapted with permission from Lodish H, Berk A, et al., eds. Molecular Cell Biology, 4th Ed, Figure 19-2, page 797. New York: W.H. Freeman and Company/Worth Publishers, 2000.

Figure 32-7: Adapted with permission from Lodish H, Berk A, et al., eds. Molecular Cell Biology, 4th Ed, Figure 19-15, page 806. New York: W.H. Freeman and Company/Worth Publishers, 2000.

Figure 32-12A: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1AO1) by Caceres-Cortes J, Sugiyama H, Ikudome K, Saito I, Wang AHJ (Interactions of cobalt(III)-pepleomycin (green form) with DNA based on NMR structural studies. Biochemistry 1997;36:9995-10005).

Figure 32-12B: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1AIO) by Takahara PM, Rosenzweig AC, Frederick CA, Lippard SJ (Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. Nature 1995;377:649–652)

Figure 32-12C: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1D10) by Frederick CA, Williams LD, Ughetto G, van der Marel GA, van Boom JH, Rich A, Wang AHJ (Structural comparison of anticancer drug/DNA complexes adriamycin and daunomycin. Biochemistry 1990;29:2538–2549).

Figure 32-13: Adapted with permission from Downing KH. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. Ann Rev Cell Dev Biol 2000;16:89–111, Figure 9.

Figure 33-3: Adapted with permission from PharmAid (copyright 2003, Jeffrey T. Joseph and David E. Golan)

Figure 34-3: Adapted from an illustration kindly provided by Professor Stephen Harrison, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School.

Figure 34-4: Adapted from Hay AJ. The action of adamantanamines against influenza A viruses: inhibition of the M2 ion channel protein. Sem Virol 1992;3:21–30, Figure 3. With permission from Elsevier.

Figure 34-9A: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 2BAT) by Varghese JN, McKimm-Breschkin JL, Caldwell JB, Kortt AA, Colman PM (The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor. Proteins 1992;14:327–332).

Figure 37-1: Adapted with permission from Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature 2002;415:674–679, Figure 2.

Figure 37-4: Adapted from www.cdc.gov/ncidod/emergplan/box23.htm

Figure 37-6: Adapted with permission from Huston CD, Haque R, Petri WA. Molecular-based diagnosis of Entamoeba histolytica infection. Expert Rev Mol Med March 22, 1999. Pages 1-11, Figure 1

Figure 39-1: Adapted with permission from Janeway CA, Travers P, Walport M, eds. Immunobiology: The Immune System in Health and Disease, 4th Ed, Figure 1.3, page 4. New York: Garland Publishing, Inc., 1999.

Figure 39-4: Adapted with permission from Janeway CA, Travers P, Walport M, eds. Immunobiology: The Immune System in Health and Disease, 4th Ed, Figure 10.11, page 378. New York: Garland Publishing, Inc., 1999.

Table 39-2: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Table 3-7. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 40-3: Adapted with permission from Serhan CS. Eicosanoids. In: Kooperman WJ, ed. Arthritis and Allied Conditions: A Textbook of Rheumatology, 14th Ed, Figure 24.2, page 516. Philadelphia: Lippincott Williams & Wilkins, 1999.

Figure 40-5: Adapted with permission from Serhan CS. Eicosanoids. In: Kooperman WJ, ed. Arthritis and Allied Conditions: A Textbook of Rheumatology, 14th Ed, Figure 24.6, page 524. Philadelphia: Lippincott Williams & Wilkins, 1999.

Figure 41-3: Adapted with permission from Janeway CA, Travers P, Walport M, eds. Immunobiology: The Immune System in Health and Disease, 4th Ed, Figure 12.12, page 474. New York: Garland Publishing, Inc., 1999.

Figure 42-1: Adapted from Cotran RS, Kumar V, Collins T, eds. Robbins Pathologic Basis of Disease, 6th Ed, Figure 14-1. Philadelphia: WB Saunders Company, 1999. With permission from Elsevier.

Figure 43-7: Adapted with permission from Fox DA. Cytokine blockade as a new strategy to treat rheumatoid arthritis: inhibition of tumor necrosis factor. Arch Intern Med 2000: 160:437-444, Figure 1.

Figure 43-8: Adapted with permission from Fox DA. Cytokine blockade as a new strategy to treat rheumatoid arthritis: inhibition of tumor necrosis factor. Arch Intern Med 2000:160:437–444, Figure 2.

Figure 43-9: Adapted from Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology, 4th Ed, Figure 8-3, page 169. Philadelphia: WB Saunders, 2000. With permission from Elsevier.

Figure 43-10: Adapted from Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology, 4th Ed, Figure 8-5, page 173. Philadelphia: WB Saunders, 2000. With permission from Elsevier.

Figure 45-2: Adapted from Barnes PJ. Asthma as an axon reflex. Lancet 1986;327:242–245. With permission from Elsevier.

Figure 45-4: Adapted with permission from Drazen JM. Treatment of asthma with drugs modifying the leukotriene pathway. N Engl J Med 1999;340:197–206, Figure 1.

Figure 48-1: Data used to render the image were deposited in the RCSB Protein Data Bank (www.rcsb.org/pdb, PDB ID: 1HXW) by Kempf DJ, Marsh KC, et al. (ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailabil-

ity in humans. Proc Natl Acad Sci USA 1995;92:2484–2488).

Figure 48-4: Adapted with permission from Schreiber SL. Target-oriented and diversity-oriented organic synthesis in drug discovery. Science 2000;287:1964–1969.

Figure 51-2: Reproduced from the CDER handbook, US Food and Drug Administration, http://www.fda.gov/cder/handbook

Figure 51-3: Reproduced from the CDER handbook, US Food and Drug Administration, http://www.fda.gov/cder/handbook

Table 51-1: Adapted with permission from Katzung BG, ed. Basic & Clinical Pharmacology, 7th Ed, Table 5-5, page 68. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998.

Table 51-2: Adapted from http://www.fda.gov/fdac/special/newdrug/testtabl.html

Table 51-3: Adapted with permission from Katzung BG, ed. Basic & Clinical Pharmacology, 7th Ed, Table 5-6, page 69. New York: Lange Medical Books/The McGraw-Hill Companies, Inc., 1998.

Figure 52-2: Adapted with permission from Price Evans DA, Manely KA, McKusick VA. Genetic control of isoniazid metabolism in man. BMJ 1960;2:485–491.

Figure 52-3: Adapted with permission from Bertilsson L, Lou YQ, Du YL, et al. Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. Clin Pharmacol Ther 1992;51:388–397. [Erratum, Clin Pharmacol Ther 1994;55:648]

# INDEX

*Note:* Page numbers followed by *f* denote figures; those followed by a *t* denote tables; those followed by *b* indicate a box.

**A**

- A bands, of myocytes, 287*f*
- Aα fibers, in pain conduction, 125*t*
- Aβ fibers, in pain conduction, 125*t*, 232
- Aδ fibers, in pain conduction, 122, 123, 125*f*, 125*t*, 232
- Aγ fibers, in pain conduction, 125*t*
- A (aminoacyl) site, of ribosome, 537, 537*f*
- AA (Alcoholics Anonymous), 260, 261*b*
- AADC (aromatic amino acid decarboxylase), in catecholamine synthesis, 162–163, 162*f*
- Abacavir, 551*f*, 563
- Abbreviated New Drug Application (ANDA), 798
- Abbreviations, for general anesthetic pharmacology, 225
- ABCA1 (ATP-binding cassette transporter A1), 362, 364*f*, 365*t*, 366
- ABCDE mnemonic, for post-myocardial infarction treatment, 391
- Abciximab, 778
  - antiplatelet activity of, 347*f*, 348, 355
  - for unstable angina, 389
- Abelcet, 583
- Absence (petit mal) seizures
  - definition of, 196*t*
  - drugs for, 201, 203–204
  - pathophysiology of, 197, 199*f*
- Absorption, 30–32
  - administration routes and, 30–32, 31*t*
  - bioavailability and, 30, 30*f*
  - of local anesthetics, 130–131
  - local factors in, 32, 32*f*
  - of oral drugs, 782
  - of toxins, 720–721, 730, 738
- Abuse, drug. *See* Addiction; Dependence; Drug(s), abuse of; Tolerance
- Acarbose, for diabetes mellitus, 465–466, 470
- ACAT (acetyl-coenzyme A:cholesterol acyltransferase), 360, 363*f*
- Accelerated development and approval status, 797
- Acceptor (aminoacyl) site, of ribosome, 537, 537*f*
- Accessory tract pathways, in cardiac conduction, 273–274, 274*f*
- ACE. *See* Angiotensin-converting enzyme (ACE)
- Acebutolol, 116, 117*t*, 119
  - for arrhythmias, 279
  - pharmacodynamics of, 387*t*
- Acetaminophen, 240, 244, 639*f*, 640, 644
  - mechanism of action of, 240*f*
  - metabolism of, 51, 721
  - site of action of, 629
  - toxication of, 721–722
  - toxicity of, 51, 731*t*, 739, 740*f*
- Acetazolamide, 305*f*, 311, 315
- Acetic acid derivatives, as NSAIDs, 639*f*, 640, 644
- Acetyl coenzyme A, in acetylcholine synthesis, 90, 91*f*
- Acetylation
  - in drug metabolism, 48*t*, 49, 51*f*, 52
  - polymorphism in, 806–807, 807*f*
- N-Acetyl-benzo-*p*-quinonimine, as acetaminophen metabolite, 721
- Acetylcholine (ACh), 89
  - action of, 67, 68, 80, 81*f*, 83, 94–96, 97*f*, 98*t*, 100–101, 101*t*
  - in catecholamine release, 109
  - degradation of, 93
    - inhibitors of, 98–100, 99*f*, 104
  - in gastric acid secretion, 684, 685*f*
  - in neural development, 96
  - receptors for
    - antagonists of, 102–103, 105
    - muscarinic, 90–92, 91*f*, 92*t*, 100–101, 100*f*, 101*t*, 104
    - nicotinic, 8, 9*f*, 90–93, 91*f*, 92*t*, 93*f*, 94*f*, 101, 102*t*, 105
  - release of, 90–91, 91*f*
    - inhibitors of, 98, 104
  - self-regulation of, 94
  - storage of, 90–91, 91*f*
    - inhibitors of, 98, 104
  - structure of, 83*f*, 100*f*
  - synthesis of, 90, 91*f*
    - inhibitors of, 98, 104
  - in vascular tone regulation, 320, 321*f*
- Acetylcholinesterase (AChE)
  - action of, 90, 91*f*, 93
  - depletion of, treatment of, 742
  - inhibitors of, 98–100, 99*f*, 104
    - toxicity of, 724, 725*f*
- Acetyl-coenzyme A:cholesterol acyltransferase (ACAT), 360, 363*f*
- *N*-Acetylcysteine
  - for acetaminophen toxicity, 739, 740*f*
  - for nitrate tolerance, 326
- *N*-Acetylglucosamine (NAG), in murein synthesis, 567, 568*f*, 569*b*
- *N*-Acetylmuramic acid (NAM), in murein synthesis, 567, 568*f*, 569*b*
- Acetylsalicylic acid. *See* Aspirin
- N-Acetyltransferase (NAT), polymorphism in, 806–807, 807*f*
- Acetyltransferases, in chloramphenicol resistance, 541
- ACh. *See* Acetylcholine (ACh)
- AChE. *See* Acetylcholinesterase (AChE)
- Acid(s), macromolecular damage from, 722
- Acid anhydrides
  - hypersensitivity to, 723
  - toxicity of, 731*t*
- Acid sensitive ion channels (ASICs), 231, 231*f*
- Acidity, pharmacokinetics and, 29, 29*f*
- Acidosis
  - in diabetes mellitus, 463
  - lactic, from biguanides, 468
  - metabolic, from diuretics, 313
- Acquired immunodeficiency syndrome. *See* Human immunodeficiency virus and infection
- Acquired (extrinsic) resistance, to antibiotics, 573
- Acquired tolerance, 250
- Acrolein, as byproduct of alkylating agents, 526
- Acromegaly, 407
- ACTH (adrenocorticotropic hormone), 404, 406*f*, 408, 409*f*, 414
  - in adrenocortical hormone regulation, 426, 426*f*, 429
  - in aldosterone regulation, 436
  - in congenital adrenal hyperplasia, 437–438, 437*f*
  - suppression of, in glucocorticoid therapy, 433
- Actin, of myocytes, 286–287, 287*f*, 288*f*, 289*t*
  - relaxation-contraction of, 318–320, 319*f*, 320*f*
- Action potentials, 63–66, 64*f*, 65*f*
  - cardiac, 268–270, 269*f*, 270*t*, 271*b*, 271*f*, 272*f*
  - definition of, 59, 63, 64*f*
  - in local anesthetic action, 128, 130*f*
  - in nociception, 122, 123*f*
  - regulation of, sodium ion channels in, 194–195, 194*f*
  - spinal cord, in pain transmission, 232, 233*f*, 234*f*
- Activated charcoal, for poisoning, 738
- Activated partial thromboplastin time assay, 350
- Active drugs, definition of, 45
- Active targeting, of tissues, for drug delivery, 786
- Active transport, 28
- Activin, 409, 444*f*, 445
- Acute adrenal insufficiency, 433
- Acute coronary syndromes, 384, 386–387
- Acute lithium intoxication, 189
- Acute mountain sickness, acetazolamide for, 312
- Acute rejection, in transplantation, 668–669, 668*t*
- Acute toxicity, 720
- Acyclovir (ACV), 547*f*, 551*f*, 552, 553*f*, 555*t*, 562
- Adalimumab, 641, 645, 675–676, 681
- Adaptive immunity, 618, 620–622, 620*t*, 621*f*, 622*f*, 659
- Addiction. *See also specific substances*
  - case study of, 247–248
  - definition of, 250
  - diagnostic criteria for, 250*b*

Addiction (*contd.*)
  drugs, list of, 248*t*
  mechanisms of, 253–255, 254*f*
  treatment of, 259–263, 260*f*, 261*b*
Addison's disease, 429–430, 433, 436
Additivity, of drugs, 605–606, 605*f*
Adefovir, 551*f*, 554, 563
Adenine
  in nucleotides, 506, 507, 509*f*, 520
  structure of, 507*t*, 520, 520*f*, 521*f*
Adeno-associated viruses, as gene therapy vectors, 766*t*, 769
Adenohypophysis
  in hypothalamic axes, 406–410, 407*f*, 409*f*
  physiology of, 404, 404*f*, 405*t*
Adenosine
  action of, 83
  for arrhythmias, 281–282
  formation of, in methotrexate therapy, 672
  for inflammation, 642
  in leukotriene inhibition, 634*f*
  as neurotransmitter, 80
  structure of, 83*f*, 507*t*, 514*f*
Adenosine deaminase, 507
  inhibitors of, 514, 514*f*
  therapy with, 773
Adenosine diphosphate (ADP)
  inhibitors of, 347*f*, 348
  in platelet activation, 336, 337*f*, 338, 338*f*, 340*f*
  in platelet aggregation, 338, 339*f*
Adenosine triphosphate (ATP)
  in cardiac contractility, 286–288, 288*f*, 290*f*
  in catecholamine release, 109, 109*f*
  as neurotransmitter, 80, 83
  as nociceptive stimuli, 231–232, 231*t*
Adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, in insulin secretion, 459–460
Adenoviruses, as gene therapy vectors, 766*t*, 768–769
Adenylate, structure of, 507*t*
Adenylyl cyclase
  in cardiac contractility, 289, 290*f*
  in drug-receptor interactions, 9, 11*f*
  in platelet aggregation, 340*f*
ADH. *See* Antidiuretic hormone (ADH, vasopressin)
Adhesion
  cell, blockade of, 679, 681
  platelet, 336, 337*f*
Adipose tissue
  distribution in, 35
  in drug half-life, 39
  as insulin target, 459, 460*f*
Adjuvants, for general anesthesia, 221
Administration routes, 30–32, 31*t*
  formulations for, 755–756, 756*t*
ADP. *See* Adenosine diphosphate (ADP)
Adrenal gland. *See also* Adrenocortical hormones
  anatomy of, 425, 426, 426*f*
  congenital hyperplasia of, 437–438, 437*f*
  in hypothalamic-pituitary-adrenal axis, 406*f*, 408, 409*f*
Adrenal medulla, anatomy of, 72, 73*f*
Adrenaline. *See* Epinephrine (EPI)
Adrenergic action. *See also* Catecholamine(s); *specific catecholamines*
  biochemistry of, 108–112, 108*f*–110*f*, 111*t*
  case study of, 107–108
  drugs acting on, 112–117, 113*f*, 117*t*, 118–119
α-Adrenergic receptors (adrenoceptors), 110, 111*t*
  agonists of, 114–115, 118–119, 381
  antagonists of, 116, 119, 173
    for hypertension, 381
    for vascular tone regulation, 322, 329, 333
$\alpha_2$-Adrenergic drugs, as agonists, 241
β-Adrenergic drugs, as antagonists, 23
β-Adrenergic receptor(s) (adrenoceptors), 110–111, 111*t*
  action of, 9, 11*t*
  agonists of, 115, 119
    for asthma, 699–700, 699*f*, 706
    for contractile dysfunction of heart, 295–296, 295*t*, 298
  antagonists of, 115–117, 117*t*, 119
    for arrhythmias, 279, 279*f*
    for congestive heart failure, 397–398, 397*t*
    for coronary artery disease, 387–388, 387*t*
    for hypertension, 380
    for hyperthyroidism, 421, 423
    overdose of, 730
    for vascular tone regulation, 329, 333
  in cardiac contractility, 289, 290*f*, 291
  gene for, polymorphism in, 805
  regulation of, 14, 15*f*
  in vascular tone regulation, 322
β-Adrenergic receptor kinase, in cardiac contractility, 291
Adrenocortical hormones, 425–439. *See also* Glucocorticoid(s); Mineralocorticoids; *specific hormones*
  androgens, 426, 426*f*, 427*f*, 437–439, 437*f*
  in congenital adrenal hyperplasia, 437–438, 437*f*
  overview of, 426, 426*f*
Adrenocorticotropic hormone (ACTH), 404, 406*f*, 408, 409*f*, 414
  in adrenocortical hormone regulation, 426, 426*f*, 429
  in aldosterone regulation, 436
  in congenital adrenal hyperplasia, 437–438, 437*f*
  suppression of, in glucocorticoid therapy, 433
Adriamycin (doxorubicin), 529, 533, 786
Adsorption, chemical, of poisons, 738
Adverse effects, reporting of, in phase IV studies, 799, 800*t*
Adverse Events Reporting System (AERS), 799
Advertising, drug, regulation of, 798–799
AEDs. *See* Antiepileptic drugs (AEDs)
Affective disorders, 179–192
  case study of, 180
  diagnostic criteria for, 184*b*
  drugs for, 186–190
    anticonvulsants, 192
    atypical antidepressants, 189
    lithium, 185, 189–190, 192
    monoamine oxidase inhibitors, 188–189, 191
    selective serotonin reuptake inhibitors, 82, 187–188, 191
    tricyclic antidepressants. *See* Tricyclic antidepressants (TCAs)
  epidemiology of, 179
  monoamine theory of, 184–185, 186*f*
  neurotransmission physiology and, 180–183, 181*f*, 182*f*, 183*t*
  pathophysiology of, 183–185, 183*b*, 184*b*, 186*f*
Affective flattening, in schizophrenia, 170
Afferent neurons, in nociception, 122
Affinity, of beta-lactams, 573
A-fibers, in pain conduction, 122
African Americans, hypertension management in, 383
Afterdepolarization, in cardiac cells, 272–273, 273*f*
Afterload
  definition of, 286
  reduction of, in congestive heart failure, 396–398
  as stroke volume determinant, 392–393, 393*f*
  in vascular tone, 318, 319*f*
Age factors
  in anesthetic induction, 216, 217*f*
  in bone mineralization, 481–482, 482*f*
  in drug metabolism, 53
  in half-life, 39
Agent Orange, toxicity of, 724
Aggregation, platelet, 337*f*, 338, 339*f*, 340*f*
Agonists, 20–21
  α-adrenergic receptor, 114–115, 118–119, 381
  $\alpha_2$-adrenergic receptor, 241
  β-adrenergic receptor, 115, 119
    for asthma, 699–700, 699*f*, 706
    for contractile dysfunction of heart, 295–296, 295*t*, 298
  aldosterone, 436
  catecholamine receptors, 114–115, 118–119
  cholinergic receptor
    muscarinic, 100–101, 100*f*, 101*t*, 104
    nicotinic, 101, 102*t*, 105
  definition of, 7, 20
  dopamine receptors, 169, 176
  in drug design, 749
  GABA$_A$ receptor, 149*t*, 150
  gonadotropin-releasing hormone (GnRH) receptors, 449, 455
  inverse, 20, 24
  irreversible, 21, 21*f*
  mineralocorticoid receptor, 436, 439
  opioid receptor, 238–239, 238*f*, 244, 263
  partial, 23–24, 23*f*
    definition of, 7, 20
    opioid receptor, 239
  serotonin receptor, 245
  types of, 26*t*
AIDS. *See* Human immunodeficiency virus and infection
Airway
  hyperreactivity of
    aspirin-induced, 639
    in asthma, 696–699, 697*f*, 698*f*
  inflammation of, in asthma, 697–698, 697*f*
D-Ala-D-Ala synthetase/ligase, in murein synthesis, 567, 568*f*
Alanine racemase, in murein synthesis, 567, 568*f*
D-Alanyl-D-alanine peptide, in murein synthesis, 567, 568*f*
Albendazole, 599, 602
Albumin
  cortisol binding to, 427
  drug binding to, 34, 34*f*
  glucocorticoid binding to, 431
Albuterol, 115, 119
  for asthma, 699, 706
  pharmacogenomics of, 805
Alcohol
  abuse of, 248*t*, 257
    cirrhosis in, 726
    pharmacologic treatment of, 260–261
    social treatment of, 260, 261*b*
    toxicity of, 729, 733*t*, 738
  avoidance of, for peptic ulcer disease, 691
  in GABA physiology modification, 155
  hyperlipidemia related to, 366–367
Alcohol dehydrogenase
  in *Entamoeba histolytica* metabolism, 595, 595*f*
  inhibitors of, 738
Alcoholics Anonymous, 260, 261*b*
Aldehyde dehydrogenase, in dopamine degradation, 164*f*
Aldesleukin, 775
Aldosterone
  actions of, 435–436
  in congestive heart failure, 394–395, 395*f*, 396*f*

  hyperfunction of, 436
  hypofunction of, 436
  metabolism of, 435
  protein binding of, 435
  receptors for, 436–437
  regulation of, 436
  synthesis of, 426*f*, 427*f*, 435
  in volume regulation, 301–303, 302*f*
Alefacept, 679
Alemtuzumab, 677, 681, 777
Alendronate, 484–485, 488
Alfentanil, 239, 244
Algal toxins, 729
Alkaline phosphatase, in bone remodeling, 475–476, 476*f*
Alkalis, macromolecular damage from, 722
Alkalosis, volume contraction, 312
Alkylamines, 652*f*
Alkylating agents, 524–527, 525*f*, 526*f*, 533, 610*t*
  for immunosuppression, 671, 673, 680
$O^6$-Alkylguanine-DNA alkyltransferase, 526
Allergy
  asthma in, 697–699, 697*f*
  histamine in, 649–650, 650*f*
  rhinitis in, 650
  to toxins, 722–724, 723*f*
Allodynia, 235
Allopurinol, 514, 710*f*, 713–715, 713*f*
Allosteric antagonists, 21*f*, 22–23, 22*f*
Allylglycine, 149*t*
Almotriptan, 245
*ALOX5* gene, for 5-lipoxygenase, polymorphism in, 805
Alpha cells, glucagon secretion from, 458
Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors. *See* AMPA receptor(s)
Alpha-glucosidase inhibitors, for diabetes mellitus, 460*f*, 465–466, 470
Alphaxalone, 150
Alprazolam, 154, 154*t*, 157
Alprostadil, 645
Alteplase, 775
Altretamine, 526, 533
Aluminum hydroxide, 486, 690, 693
Alveolar macrophages, in fungal defense, 580
Alveolar partial pressure ($P_{alv}$), of anesthetics, 207, 210–217, 214*f*–216*f*
Alveolar ventilation, inhaled anesthetics and, 211–212, 212*f*
Alzheimer's disease
  acetylcholine deficiency in, 96
  acetylcholinesterase inhibitors for, 100
  gene therapy for, 763*t*, 764
Amantadine, 547*f*, 548–549, 550*f*, 562
  action of, 157
  for Parkinson's disease, 147, 169, 176
Ambenonium, 100, 104
Ambisome, 583
Amebiasis. *See Entamoeba histolytica*
Amide(s), as NSAIDs, 639*f*, 640, 644
Amide-linked local anesthetics, 124, 126*f*, 131, 131*f*, 133–135
Amidophosphoribosyltransferase (amidoPRT), in purine synthesis, 710, 710*f*
Amifostine, 527
Amikacin, 539, 543
Amiloride
  action of, 307*f*
  for hypertension, 313–314, 316, 380
Amino acid(s)
  as neurotransmitters, 80, 83*f*. *See also* Gamma-aminobutyric acid (GABA); Glutamate, Glycine
    actions of, 140, 140*f*
  in protein structures, 4–5, 4*f*
  transport across blood–brain barrier, 86
Aminoacyl (A) site, of ribosome, 537, 537*f*
γ-Aminobutyric acid. *See* Gamma-aminobutyric acid (GABA)
Aminoglutethimide, 435, 435*t*, 439
  as aromatase inhibitor, 449–450, 455
  site of action of, 427*f*
Aminoglycosides, 537*f*, 539–540, 539*t*, 540*f*, 543
  beta-lactam synergy with, 573–574, 607
Aminophylline, 700
Amiodarone, 280–281, 281*t*, 421–422
Amisulpride, 174
Amitriptyline, 118, 191
  action of, 114
  for pain, 241, 245
Amlodipine
  for coronary artery disease, 388
  for hypertension, 381
  for vascular tone regulation, 327–328, 332
Ammonia, toxicity of, 726
Amobarbital, 152*t*, 159
Amoxicillin, 573, 574, 577, 691
AMPA receptor(s)
  in amyotrophic lateral sclerosis, 147
  antagonists of, 145
  in epilepsy, 145, 147
  in hyperalgesia, 146*f*
  physiology of, 141–142, 142*f*, 143*t*
  receptor interactions of, 146*f*
  in stroke, 145, 145*f*
AMP-dependent protein kinase, biguanide activation of, 468
Amphetamines, 118
  action of, 113–114, 257–259, 258*f*
  addiction to, 257–259, 258*f*
  schizophrenia-like state due to, 171
Amphotec, 583
Amphotericin B, 581*f*, 582–583, 585, 596
  flucytosine with, 607
  liposomal formulation of, 786
Ampicillin, 573, 574, 577
Amplicons, in herpesviruses, 769
Amprenavir, 556, 564
Amps, in Ohm's law, 60, 60*f*
Amrinone
  for congestive heart failure, 397*t*, 398
  inotropic effects of, 296–298
  for vascular tone modulation, 326
Amsacrine, 522*f*, 529, 534
Amygdala, 76
  dopamine receptors in, 164, 165*f*
Amyl nitrate, toxicity of, 724, 731*t*
Amyl nitrite, for cyanide poisoning, 739, 739*f*
Amylase, therapy with, 773
Amyotrophic lateral sclerosis
  gene therapy for, 764
  glutamate receptors in, 147
Anabolic agents, bone, 485–486, 489
Anabolic hormones, insulin as, 462
Anabolic steroids
  hepatotoxicity of, 726
  toxicity of, 731*t*
Anakinra, 641, 645, 676, 681, 778
Analgesia stage, of general anesthesia, 206*f*
Analgesic(s), 229–245
  adrenergic agonists, 241
  vs. anesthetics, 123
  antiarrhythmic agents, 241, 245
  anticonvulsants, 241, 245
  antidepressants, 241, 245
  case study of, 229–230
  for migraine, 241–242, 245
  NMDA receptor antagonists, 241, 245
  NSAIDs, 239–241, 240*f*, 241–242
  opioid receptor agonists, 238–239, 238*f*, 244
  pain pathophysiology and, 234–237, 235*f*–237*f*
  pain physiology and, 230–233, 231*f*, 231*t*, 233*f*, 234*f*
  summary of, 244–245
Analgesic index, of general anesthetics, 207
Analytic toxicology, 719
Anandamide, 233
Anaphylaxis, histamine in, 651
Anastrozole, 449*f*, 450, 455, 610*t*
ANDA (Abbreviated New Drug Application), 798
Androgens. *See also* Testosterone
  abuse of, 454
  actions of, 442
  adrenocortical, 437–438, 437*f*, 439
  in calcium homeostasis, 479
  deficiency of, 448
  excess of, in polycystic ovarian syndrome, 447–448
  metabolism of, 442, 444*f*
  receptor for, antagonists of, 449*f*, 451–452, 455
  replacement of, 454
  synthesis of, 442, 443*f*, 445, 445*f*
Androstenedione, 438, 443*f*
Anemia
  from amphotericin B, 583
  aplastic, from chloramphenicol, 541
  definition of, 658
  erythropoietic agents for, 662–662, 665
  hemolytic, autoimmune, 670*t*
  in lead poisoning, 730
Anergy, from costimulation inhibitors, 677–679, 678*f*
Anesthetics
  vs. analgesics, 123
  epidural, 132
  gaseous, absorption of, 32
  general. *See* General anesthetics
  intravenous, 220–221, 220*f*, 227–228
  local. *See* Local anesthetics
  spinal (intrathecal), 132
Angina (pectoris), 318
  pathophysiology of, 385, 385*f*
  rest, 326
  stable, 388–389
    clinical management of, 387–388
    pathophysiology of, 385, 385*f*
  unstable, 326
    clinical management of, 389–390
    pathophysiology of, 385, 385*f*, 386
  variant
    clinical management of, 387
    pathophysiology of, 385, 385*f*
  vasospastic, 388
Angioedema, from angiotensin-converting enzyme inhibitors, 310
Angiotensin(s)
  in adrenocortical hormone regulation, 426, 426*f*
  in vascular tone regulation, 322
  in volume regulation, 301–303, 302*f*
Angiotensin receptor(s)
  antagonists of
    action of, 323*f*
    for hypertension, 382
    for vascular tone regulation, 322, 333
    for volume regulation, 311, 315
  in volume regulation, 302–303
Angiotensin-converting enzyme (ACE)
  action of, 310, 310*f*
  in volume regulation, 301–302, 302*f*

Angiotensin-converting enzyme inhibitors
  action of, 323f
  for congestive heart failure, 396–397, 397t
  for hypertension, 382
  nephrotoxicity of, 727
  for vascular tone regulation, 322, 333
  for volume regulation, 310–311, 310f
Angiotensinogen, in volume regulation, 301, 302f
Aniline dyes, toxicity of, 731t
Anilo-phenylpiperidine, 239
Animal studies
  in drug development, 754
  in preclinical evaluation, 794
Anisoylated plasminogen streptokinase activator complex (APSAC), 354, 356, 390
Ann Arbor staging system, for Hodgkin's disease, 612t
*Anopheles* mosquito, as malaria vector, 588, 588f
Antacids, 16, 690, 693
Antagonism, in drug interaction, 605–606, 605f, 608
Antagonists, 21–23, 21f, 22f
  acetylcholine receptor, 102–103, 105
  α-adrenergic receptor, 23, 116, 119, 173, 322, 329, 333, 381
  β-adrenergic receptor. *See under* β-Adrenergic receptor(s) (adrenoceptors)
  allosteric, 21f, 22–23, 22f
  AMPA receptor, 145
  androgen receptor, 449f, 451–452, 455
  angiotensin receptor. *See under* Angiotensin receptor(s)
  antidiuretic hormone, 311
  catecholamine receptor, 115–117, 119
  chemical, 21, 21f, 23
  cholinergic receptor, 173
  competitive, 7, 21–22, 21f, 22f
  dopamine receptor, 171–175, 172f, 173f, 177–178
  endothelin receptor, 322, 323f
  GABA receptor, 149t, 158
  glucocorticoid receptor, 435, 439
  glutamate receptor, 157
  glycoprotein IIB-IIIa, 348, 390
  heparin, 350
  histamine receptor
    H1, 651–652, 652f, 654
    H2, 652, 653f, 654
    H3 receptor, 652
  leukotriene receptor, 643, 646
  mineralocorticoid receptor, 436–437, 439
  NMDA receptor, 145, 241, 245
  noncompetitive, 7, 21f, 22–25, 22f, 25f
  nonreceptor, 21, 21f, 23
  opioid receptor, 238, 244, 263. *See also* Naloxone; Naltrexone
  physiologic, 21, 21f, 23
  progesterone receptor, 449f, 452, 456
  thromboxane receptor, 642, 646
  for toxicity, 741–742
  types of, 7, 21, 21f, 26t
  uncompetitive, 7
Anterior pituitary gland
  in hypothalamic axes, 406–410, 407f, 409f
  physiology of, 404, 404f, 405t
Anthracyclines, 522f, 529, 533, 610t
Antiadrenergic effects, of tricyclic antidepressants, 187
Antianginal drugs, 389–390. *See also specific drugs*
Antiarrhythmic agents, 274–284
  adenosine, 281–282, 284
  case study of, 267–268
  class I (fast sodium ion channel blockers), 275–278, 276f, 277f, 283
  class IA, 276–278, 277f, 283
  class IB, 277f, 278, 283
  class IC, 277f, 278, 283
  class II (beta-adrenergic blockers), 279, 279f, 283
  class III (repolarization inhibitors), 279–281, 279f, 281t, 284
  class IV (calcium channel blockers), 281, 281f, 284
  mechanisms of action of, 274–275
  for pain, 241, 245
  potassium, 282
Antibiotics. *See* Antimicrobial drugs; *specific drugs*
Antibody(ies)
  digoxin, 294, 741
  as drugs, 752–753
  in immune complexes, 723f, 724
  as immunosuppressive drugs, 676–677, 681
  monoclonal, 760, 777–779
    for asthma, 703
    for cancer, 610t
    for immunosuppression, 677, 681
  polyclonal, for immunosuppression, 676–677, 681
Antibody-dependent cytotoxic hypersensitivity, 723, 723f
Anticancer drugs. *See* Antineoplastic drugs
Anti-CD25 monoclonal antibodies, for immunosuppression, 677, 681
Anti-CD52 monoclonal antibodies, for immunosuppression, 677, 681
Anticholinergic agents
  for asthma, 700, 706
  disopyramide, 277–278
  for peptic ulcer disease, 690, 694
  quinidine, 276–277
  tricyclic antidepressants, 187
Anticipation, in genetics, 763
Anticoagulants, 348–353, 355. *See also specific anticoagulants, e.g.,* Heparin
  delayed-acting, oral, 350–353, 351f, 352t
  direct thrombin inhibitors, 353
  endogenous, in hemostasis regulation, 340
  immediate-acting, 348–350, 349f
  recombinant activated protein C, 353
  toxicity of, 742–743
Anticonvulsants. *See also* Antiepileptic drugs
  for bipolar disorder, 185
  for pain, 241, 245
Antidepressants
  action of, 82, 185, 186f
  atypical, 189, 191, 192
  autoreceptor down-regulation due to, 185, 186f
  for bipolar disorder, 185
  case study of, 180
  for drug addiction, 262
  monoamine oxidase inhibitors, 188–189
  neurotransmission and, 180–183, 181f, 182f, 183t
  for pain, 241, 245
  selective serotonin reuptake inhibitors, 82, 187–188, 191
  tricyclic. *See* Tricyclic antidepressants (TCAs)
Antidigoxin antibodies, 294, 741
Antidiuretic hormone (ADH, vasopressin), 410, 411f
  antagonists of, 311
  in volume regulation, 303f, 304
Antidotes, for toxins, 731t–738t
Antiepileptic drugs (AEDs)
  acting at T-type calcium ion channel, 201, 203
  benzodiazepines as, 154, 154t
  case study of, 193–194
  enhancing GABA-mediated inhibition, 201–202, 204
  mediating sodium ion channel inhibition, 198–201, 200f, 203
  new, 204
Antifungal drugs, 604
  in combination therapy, 607
  ergosterol synthesis inhibitors, 581–582, 581f, 585
  ergosterol-binding compounds, 582–583, 585
  ideal, 580–581
  microtubule inhibitors, 531
  targets of, 498
  thymidylate synthase inhibitors, 583, 584f, 585
  types of, 498
Antigen(s)
  allergens as, in asthma, 697–698, 697f
  in immune complexes, 723f, 724
  major histocompatibility complex, 620–621, 621f
Antigen-presenting cells (APCs), 618–620, 621f, 622f, 625
Anti-helminthic agents, 598–599, 602
Antihistaminergic effects, of tricyclic antidepressants, 187
Antihistamines, 651–654
  case study of, 647
  H1 receptor antagonists, 651–652, 652t, 654
  H2 receptor antagonists, 652, 653t, 654
  H3 receptor antagonists, 652
  strategies for, 651, 651t
Antihypertensive agents
  ACE inhibitors, 382
  α-adrenergic antagonists, 116–117, 117t, 119
  β-adrenergic antagonists, 116–117, 117t
  α-adrenergic receptor agonists, 115
  α-adrenergic receptor antagonists, 329, 333
  β-adrenergic receptor antagonists, 329, 333
  angiotensin receptor antagonists, 311, 379t, 382
  angiotensin-converting enzyme inhibitors, 310–311, 310f, 315, 322, 333
  calcium channel blockers, 327–328, 328f, 328t, 332
  clonidine, 115
  demographic factors affecting, 383
  diuretics, 379–380, 379t
    potassium-sparing, 313–314, 316
    thiazide, 313, 316
  hydralazine, 329, 333
  for hypertensive crisis, 383
  monotherapy with, 376, 382
  neurohormonal modulators, 382
  potassium ion channel openers, 328–329, 332
  renin-angiotensin–aldosterone system blockers, 309–311, 310f, 315, 330, 333, 379t
  sodium nitroprusside, 324, 324f, 325f, 331
  stepped care with, 382–383
  thiazide diuretics, 313, 316
  vasodilators, 379t
Anti-inflammatory agents. *See also* Eicosanoids, inhibitors of
  for asthma, 701–703, 702f, 707
  case study of, 627–628
  glucocorticoids, 428, 429f, 432
Antimalarial drugs, 498, 590–593, 600
  electron transport chain inhibitors, 590f, 592–593, 600
  heme metabolism inhibitors, 590–592, 591f, 592f, 600

Antimetabolites, 610*t*
  case study of, 505–506
  definition of, 505
  folate metabolism inhibitors, 509–513, 510*f*, 511*t*, 512*b*, 517
  for immunosuppression, 671–673, 671*f*–673*f*, 672*t*, 680
  nucleotide synthesis and, 506–509, 506*f*, 507*t*, 508*f*–511*f*
  purine metabolism inhibitors, 513–514, 514*f*, 517
  ribonucleotide reductase inhibitors, 514–515, 517
  "rogue" nucleotides in, 515, 516*f*, 518
  sites of action of, 506*f*
  thymidylate synthase inhibitors, 513, 513*f*, 517
Antimicrobial drugs
  antagonistic effects of, 496
  antimetabolite. *See* Antimetabolites
  antiviral. *See* Antiviral drugs
  classes of, 495–496, 496*f*
  in combination therapy, 503, 603–608
    beta-lactam antibiotics with beta-lactamase inhibitors, 607
    DHFR inhibitors with sulfonamides, 512–513
    drug interactions in, 605–606, 605*f*
    minimum inhibitory concentration and minimum bactericidal concentration and, 604–605, 604*f*, 604*t*, 605*f*
    for polymicrobial infections, 607–608
    synergistic, 606–607
    for tuberculosis, 606
    unfavorable combinations in, 608
  dihydrofolate reductase inhibitors, 510*f*, 511–513, 511*t*, 512*b*, 517
  dose for, in kidney failure, 40*b*
  nephrotoxicity of, 727
  overprescription of, 503
  pathogen characteristics and, 495–498, 496*f*, 497*f*
  for peptic ulcer disease, 691
  prophylactic, 503
  purine metabolism inhibitors, 513–514, 514*f*, 517
  resistance to. *See* Resistance, drug
  ribonucleotide reductase inhibitors, 514–515, 517
  with "rogue" nucleotide structures, 515, 516*f*, 518
  selective targeting mechanisms of, 494–495, 494*t*
  targets of, 495–496, 496*f*
  thymidylate synthase inhibitors, 513, 513*f*, 517
  topoisomerase inhibitors, 529
  transcription inhibitors, 538, 544
  translation inhibitors, 538–544, 539*t*, 540*f*
  treatment methods for, 503–504
Antimony compounds, 596, 601
Antimycobacterial agents, 575, 578, 606
Antineoplastic drugs
  alkylating agents, 524–527, 525*f*, 526*f*, 533, 610*t*
  antimetabolite. *See* Antimetabolites
  antineutropenic agents with, 655–656
  bleomycins, 527–528, 528*f*, 533, 610*t*
  cancer cell characteristics and, 498–500, 500*f*, 501*f*, 609
  cell cycle interactions of, 499–500, 501*f*
  classes of, 499, 500*f*
  in combination therapy, 503
    dosing regimens in, 611
    drugs used in, 610*t*
    general considerations in, 609, 611, 611*t*
    for Hodgkin's disease, 611–612, 612*t*
    rationale for, 611
    synergism in, 611
    for testicular cancer, 612, 613*f*
  dihydrofolate reductase inhibitors, 510*f*, 511–513, 511*t*, 512*b*, 517
  interferons, 663, 666
  levamisole, 663–664, 666
  microtubule inhibitors, 530–531, 530*f*, 534
  monoclonal antibodies, 760
  platinum compounds, 527, 527*f*, 528*f*, 533, 610*t*
  polymer-based delivery of, 785
  prophylactic, 503
  purine metabolism inhibitors, 513–514, 514*f*, 517
  for refractory or recurrent disease, 612–613
  resistance to, 500–503, 502*t*, 609, 610*t*, 611, 611*t*
  ribonucleotide reductase inhibitors, 514–515, 517
  with "rogue" nucleotide structures, 515, 516*f*, 518
  selective targeting mechanisms of, 494–495, 494*t*
  thymidylate synthase inhibitors, 513, 513*f*, 517
  topoisomerase inhibitors, 522*f*, 528–530, 533–534
  treatment methods for, 503–504
Antineutropenic agents, 662–663, 665
Antiparasitic drugs, targets of, 498
α-Antiplasmin, 343, 343*f*
Antiplatelet agents
  ADP receptor pathway inhibitors, 348
  cyclooxygenase inhibitors, 345, 346*f*, 347*f*
  glycoprotein IIb-IIIa antagonists, 348
  nitrates, 326
  phosphodiesterase inhibitors, 345, 347*f*, 348
  salicylates, 638–640, 639*f*, 644
Antiprotozoal agents, 595–596, 601
Antipseudomonal penicillins, 575
Antipsychotic drugs
  atypical, 174–175, 178
  D2 receptor affinity of, 171, 172*f*
  definition of, 171
  drug interactions of, 174
  mechanism of action of, 171–172, 172*f*, 173*f*
  vs. neuroleptic drugs, 171
  pharmacokinetics of, 174
  side effects of, 172–173
  structures of, 172, 173*f*
  typical, 171–174, 173*f*, 177
Antiretroviral drugs
  development of, 747–748, 748*f*
  targets of, 497–498, 497*f*
Antisense strategies, in gene therapy, 770–771, 770*f*
Antistaphylococcal penicillins, 574
Antithrombin III
  defects of, 345
  in hemostasis regulation, 341, 342*f*
  in heparin action, 349–350, 349*f*
Antithrombotic mechanisms, 336
Anti-thymocyte globulin (ATG), for immunosuppression, 676–677, 681
Antivenins, 752*t*
  action of, 741
  hypersensitivity to, 724
  toxicity of, 731*t*
Antiviral drugs, 547–564
  attachment and entry inhibitors, 547–548, 549*f*, 562
  coating inhibitors, 548–549, 562, 550*f*
  in combination therapy, for HIV, 608
  genome replication inhibitors, 549–556, 562–564
    DNA polymerase inhibitors, 555, 557*f*, 563
    for hepatitis B, 551*f*, 554–555, 563
    for herpesvirus infections, 551*f*, 552–554, 553*f*, 562
    for HIV infection, 551*f*, 554–555, 555*t*, 556*b*, 563
    non-nucleoside DNA polymerase inhibitors, 555, 557*f*, 563
    reverse transcriptase inhibitors, 555–556, 556*b*, 557*b*, 557*f*, 564
  maturation inhibitors, 547*f*, 556–557, 558*f*, 559*b*, 564
  targets of, 496–498, 497*f*
  with unknown mechanisms of action, 560, 564
  viral release inhibitors, 547*f*, 557, 559–560, 559*f*, 564
Anxiolytics, benzodiazepines as, 154, 154*t*
APCs (antigen-presenting cells), 618–620, 621*f*, 622*f*, 625
Aplastic anemia, from chloramphenicol, 541
ApoAI, 359, 360*t*, 361, 361*f*
  defects of, 365*t*, 366
ApoB48, 359, 360*t*, 361*f*
ApoB100 defects, 365, 365*t*
ApoCII, 359, 360, 360*t*, 361*f*
  deficiency of, 365*t*, 366
ApoE
  defects of, 365*t*, 366
  polymorphism in, 806
Apolipoproteins, 358*t*, 359, 359*f*, 360, 360*t*
  polymorphism in, 806
Apomorphine
  as poison antidote, 738
  schizophrenia-like state due to, 171
Apoptosis
  of cancer cells, 499
  defects of, 728, 764
  insensitivity to, 502–503
  in toxicity, 722, 725–726
Apoptotic bodies, in toxicity, 726
Approval, of new drugs. *See under* New drug evaluation
Aprobarbital, metabolism of, 152*t*
APSAC (anisoylated plasminogen streptokinase activator complex), 354, 356, 390
Aquaporins, in volume regulation, 303*f*, 304, 305, 307
Arabinogalactan, in mycobacterial cell wall, 566*f*, 569
Arabinosyl transferase, in mycobacterial cell wall synthesis, 569
AraC (cytarabine, cytosine arabinoside), 506*f*, 510*f*, 515, 516*f*, 518
Arachidonic acid
  generation of, 628, 628*f*
  metabolism of
    cyclooxygenase pathway for, 628–631, 628*f*–630*f*, 629*t*, 631*t*, 632*f*
    epoxygenase pathway for, 628*f*, 633
    isoprostane formation in, 628*f*, 633
    lipoxygenase pathway for, 628*f*, 631–633, 633*t*, 634*f*, 635*f*
    pathways for, 628, 628*f*, 633, 636, 636*f*, 636*t*
  prostaglandin synthesis from, 345, 346*f*
  release of, in platelet activation, 336
  structure of, 628, 628*f*
2-Arachidonylglycerol, 233
Arcuate nucleus, dopaminergic neurons in, 166
Area postrema, dopaminergic neurons in, 166

- Argatroban, 353
- Aripiprazole, 178
- Aromatase, 442, 443*f*
  - inhibitors of, 449–450, 449*f*, 455
- Aromatic amino acid decarboxylase
  - in catecholamine synthesis, 108, 108*f*
  - in serotonin synthesis, 180, 181*f*
- Aromatic amino acid decarboxylase (AADC, DOPA decarboxylase), in catecholamine synthesis, 162–163, 162*f*
- Arrhythmia, cardiac. *See* Antiarrhythmic agents; Cardiac arrhythmia(s)
- Arsenic compounds, toxicity of, 726, 731*t*
- Artemisinin *(quinghao)*, 592, 592*f*, 600
- Arterial partial pressure ($P_{art}$), of anesthetics, 213, 214, 215*f*, 216
- Arteriolar tone, 318, 319*f*
- Artery(ies), endothelial injury in, thrombosis in, 343
- Arthritis
  - gouty, 709, 711–712, 711*t*
  - intra-articular glucocorticoid injections for, 434
  - rheumatoid. *See* Rheumatoid arthritis
- Aryl hydrocarbon receptor, dioxin binding to, 724
- Asbestos
  - inhalation of, 721
  - pulmonary fibrosis from, 726
  - toxicity of, 731*t*
- Ascariasis, drugs for, 598–599, 602
- Ascites, formation of, 308–309, 309*f*
- Ascorbic acid, in catecholamine synthesis, 162, 162*f*
- Asian population, hypertension management in, 383
- ASICs (acid sensitive ion channels), 231, 231*f*
- L-Asparaginase, therapy with, 775
- Aspartate
  - as neurotransmitter, 80
  - structure of, 83*f*
- Aspirin, 240, 244, 638–639, 639*f*, 644
  - antiplatelet activity of, 345, 347*f*, 355
  - asthma induced by, 639, 702, 702*f*
  - for coronary artery disease, 389
  - in irreversible enzyme inactivation, 53
  - as noncompetitive antagonist, 23
  - for unstable angina, 389
- Aspirin-induced airway hyperactivity, 639
- Aspirin-triggered lipoxins (ATLs), 639, 643
- Assembly, in viral life cycle, 546, 547*f*, 548*f*
- Astemizole, 652, 654
- Asthma, 695–707
  - β-adrenergic receptor antagonist avoidance in, 388
  - aspirin-induced airway hyperactivity in, 639, 702, 702*f*
  - case study of, 695–696
  - definition of, 696
  - drugs for, 699–707
    - β-adrenergic receptor agonists, 115
    - aerosol, 703–704
    - anti-inflammatory agents, 701–703, 702*f*, 706
    - bronchodilators, 699–700, 699*f*, 706
    - controllers, 699
    - delivery of, 703–704, 707–708
    - glucocorticoids, 425–426
    - relievers, 699
    - vs. severity, 704–705, 704*t*
  - histamine sensitivity in, 648, 648*t*
  - inflammation in, 637
  - mild intermittent, 704, 704*t*
  - mild persistent, 704, 704*t*
  - moderate persistent, 704, 704*t*
  - pathophysiology of, 696–699, 697*f*, 698*f*
  - pharmacogenomics of, 805
  - severe persistent, 704, 704*t*
- Astroglia, 85
- Atenolol, 117, 117*t*, 119
  - for arrhythmias, 279, 283
  - for hypertension, 380
  - pharmacodynamics of, 387*t*
  - for vascular tone regulation, 333
- ATG (anti-thymocyte globulin), 676–677, 681
- Atherosclerosis
  - abnormal blood flow in, thrombosis in, 343
  - of coronary arteries. *See* Coronary artery disease (CAD)
  - dyslipidemia and, 357, 361, 363*f*
  - foam cells in, 361, 362*f*, 363*f*
  - in HDL metabolic disorders, 365*t*, 366
  - plaque rupture in, 385*f*, 386
- Atorvastatin, 369, 373, 747
- Atovaquone, 590, 593, 600, 797
- ATP. *See* Adenosine triphosphate (ATP)
- ATPase, in dopamine transport, 163, 163*f*
- ATP-binding cassette transporter A1 (ABCA1), 362, 364*f*
  - defects of, 365*t*, 366
- Atracurium, 228
- Atria, volume sensing in, 300–301
- Atrial depolarization, P wave representing, 271*b*, 272*f*
- Atrial fibrillation, 276*b*, 293
- Atrial flutter, 276*b*, 277
- Atrioventricular node
  - dysfunction of
    - beta-blocker effects on, 278, 278*f*
    - calcium channel blocker effects on, 281, 281*f*
  - function of, 270–271, 273–274
  - pacemaker cells in, 268
  - quinidine effects on, 276–277
- Atropine, 105
  - for acetylcholinesterase depletion, 742
  - for asthma, 700
  - clinical applications of, 101
  - toxicity of, 731*t*
- Attachment, in viral life cycle, 546, 547*f*, 548*f*
- A-type natriuretic peptide, in volume regulation, 303, 303*f*
- Atypical antidepressants, 189, 192
- Atypical antipsychotics, 174, 178
- Augmentin, 574
- Aura, before seizure, 196
- Autacoids. *See also* Arachidonic acid; Eicosanoids; *specific substances*
  - definition of, 627
- Autoimmune diseases
  - diabetes mellitus type I, 463–464, 463*t*
  - examples of, 669, 670*t*
  - glucocorticoids for, 432
  - immunosuppressive drugs for. *See* Immunosuppressive drugs
  - pathophysiology of, 669, 670*t*
  - from toxins, 722, 724
- Autolysins, of bacterial cell wall, 569–570
- Automaticity, 268
  - altered, 272
  - calcium channel blocker effects on, 328*f*, 328*t*
  - sodium ion channel blocker effects on, 275, 276*f*
- Autonomic nervous system
  - anatomy of, 72–73, 74*f*
  - catecholamine action in. *See* Catecholamine(s)
  - cholinergic transmission effects on, 94–95, 97*f*, 98*t*
  - muscarinic cholinergic transmission in, 91–92, 92*t*
  - neuronal organization of, 78
  - neurotransmitters in, 81*f*
  - in vascular tone regulation, 322
- Autoreceptors, 67, 185, 186*f*
- Autoregulation, of vascular tone, 322
- Avolition, in schizophrenia, 170
- Axis, endocrine. *See* Endocrine axes
- 5-Azacytidine, 515, 516*f*, 518, 662
- Azathioprine, 513, 514*f*, 517
  - allopurinol interactions with, 713–714, 713*f*
  - for immunosuppression, 671–672, 671*f*, 680
- Aztreonam, 575, 578
- Azidothymidine (zidovudine), 547*f*, 551*f*, 554–555, 555*t*, 563, 608
- Azithromycin, 541, 544
- Azole drugs, 580–582, 581*f*, 585
- AZT (zidovudine), 547*f*, 551*f*, 554–555, 555*t*, 556*b*, 563, 608

**B**

- B cells, 620*t*, 622, 622*f*, 656*t*
  - costimulation by, inhibitors of, 678
  - function of, 659
  - production of, 659–661
- *Bacillus cereus*, toxins of, 729
- Bacitracin, 571–572, 577
- Baclofen, 149*t*
  - GABA receptor interactions with, 149
- Bacteria. *See also* Antimicrobial drugs; *specific bacteria*
  - drug targets in, 495–496, 496*f*. *See also* Bacterial cell wall synthesis
  - intracellular, 573
- Bacterial cell wall synthesis, 566–570
  - crosslinking in, 567, 568*f*, 569, 569*b*, 570*f*
  - degradation and, 569–570
  - enzymes in, 567, 568*f*, 569*b*
  - functional aspects of, 566–567, 566*f*
  - inhibitors of, 570–578
    - case study of, 565–566
    - crosslinking, 572–575, 572*f*, 574*f*, 577–578
    - murein monomer, 570–572, 571*f*, 577
    - murein polymerization, 570–572, 577
    - mycobacterial, 575, 578
  - murein monomers, 567, 568*f*, 569*b*
  - mycobacterial, 569, 571*f*
  - polymerization in, 567, 568*f*
  - structural aspects of, 566–567, 566*f*
- Bactericidal drugs, 496, 604–605, 604*f*, 604*t*, 605*f*
  - aminoglycosides, 539, 540*f*
  - beta-lactams as, 573
- Bacteriostatic drugs, 496, 604–605, 604*f*, 604*t*, 605*f*
- Bactoprenol, in murein synthesis, 567, 568*f*
- BAL (British anti-lewisite, dimercaprol), 741, 741*f*
- Balanced anesthesia, 221
- Barbiturates, 150–153, 159. *See also specific drugs*
  - abuse of, 248*t*, 255–256
  - action of, 149*t*, 150–151, 151*f*, 151*t*, 200*f*
  - cerebral cortical effects of, 75
  - clinical applications of, 151–152, 152*t*
  - dependence on, 153
  - GABA receptor binding site for, 148*f*
  - intravenous, pharmacology of, 220
  - metabolism of, 152, 152*t*
  - pharmacokinetics of, 152, 152*t*
  - for seizures, 202, 204
  - tolerance to, 153
  - toxicity of, 153
- Baroreceptors, in volume regulation, 301
- Barriers, physiologic, 28–29, 29*f*

- Basal ganglia, 75–76, 75f
  - dopamine receptors in, 164, 165f
- Basal nucleus of Meynert, 79–80, 79t
- Basic multicellular units, in bone remodeling, 475
- Basiliximab, 677, 681, 778
- Basophils, 619, 619f, 620t, 656t, 659
- BCNU (carmustine), 525f, 526, 533, 786
- BCR-Abl kinase, imatinib interaction with, 5–6, 6f, 10
- BDNF (brain-derived neurotrophic factor), 232, 236, 236f
- Beclomethasone, 439
  - for asthma, 701, 707
  - inhaled, 433
  - structure of, 434f
- Befloxatone, 114, 118, 188, 191
- Belladonna alkaloids, toxicity of, 742
- Benazepril, 315
- Bendroflumethiazide, 316
- Benign prostatic hyperplasia
  - α-adrenergic antagonists for, 116
  - 5α-reductase inhibitors for, 448
- Benzene
  - inhalation of, 721
  - toxicity of, 720, 731t
  - toxification of, 721
- Benzo[a]pyrene, as carcinogen, 727, 727f
- Benzocaine, toxicity of, 732t
- Benzodiazepines, 153–155, 157–158. *See also specific drugs*
  - abuse of, 248t, 255–256
  - action of, 149t, 153, 153f, 200f
  - cerebral cortical effects of, 75
  - clinical applications of, 154–155, 154t
  - dependence on, 155
  - GABA receptor binding site for, 148f
  - ion channel conductance and, 8
  - pharmacokinetics of, 155
  - for seizures, 202, 204
  - tolerance to, 155
  - toxicity of, 155, 742
- Benzothiazepines, 327
- Benzothiazines, 240
- Benztropine, 169–170
- Beryllium
  - pulmonary fibrosis from, 726
  - toxicity of, 732t
- Beta cells
  - destruction of, in diabetes mellitus, 463–464
  - insulin secretion from, 458
- Beta-blockers. *See* β-Adrenergic receptors (adrenoceptors), antagonists of
- Beta-glucocerebrosidase, therapy with, 758, 773
- Beta-lactam antibiotics, 566, 568f, 572–575. *See also specific antibiotics, e.g.,* Penicillin(s)
  - resistance to, 573
  - spectrum of action of, 573
  - structures of, 572–573, 572f
  - synergism of, 573–574
  - toxicity of, 574, 574f
- Beta-lactam ring, 572, 572f
- Beta-lactamases, 573
  - inhibitors of, 573. *See also* Beta-lactam antibiotics
    - penicillins with, 607. *See also specific combinations*
- Betaxolol, pharmacodynamics of, 387t
- Bethanechol, 104
  - action of, 101, 101t
  - structure of, 100f
- Bezafibrate, 370
- B-fibers, in pain conduction, 122, 125t
- Bicarbonate
  - for peptic ulcer disease, 690, 693
  - reabsorption of, in volume regulation, 305, 305f
  - in stomach, protective effects of, 685
- Bicuculline, 149t, 150
- Bier's block, 132
- Big endothelin, 321, 321f
- Biguanides, for diabetes mellitus, 460f, 468, 469t, 471
- Bile acid absorption inhibitors, for dyslipidemia, 367–368, 373
- Biliary excretion, 36–38, 37f
- Bilirubin, conjugation of, 49
- Bimatoprost, 645
- Binders, for pills, 756
- Binding
  - drug-receptor, 5–6, 6f, 8, 10, 18–19, 18f
  - of local anesthetics, 131
  - plasma protein, 34, 34f
- Binding site, of amino acids, 5
- Bioavailability, 30, 30f
- Biochemical assays, in drug development, 753, 753t
- Bioequivalence, of generic drugs, 798
- Biogenic amine neurotransmitters, 80–83, 83f, 84f
- Biological characterization, in drug development, 753–754, 753t
- Biopharmaceutical review, of new drugs, 796, 797f
- Biotransformation
  - definition of, 45
  - phase I reactions in, 35, 45–49, 46t–47t, 50f
  - phase II reactions in, 35, 45–47, 48t, 49, 51f
- Bipolar disorder
  - case study of, 180
  - definition of, 183, 184
  - diagnostic criteria for, 184b
  - drugs for, 180, 185, 189–190, 192
  - neurotransmission and, 180–183, 181f, 182f, 183t
  - pathophysiology of, 183–185
- Birth defects, from teratogens, 447b, 722, 728
- *Bis*-alkylation, 524
- Bismuth, colloidal, 690–691, 694
- Bisoprolol
  - for arrhythmias, 279
  - pharmacodynamics of, 387t
- Bisphosphonates
  - for bone disorders, 484–485, 485f, 488
  - for hypercalcemia, 487
- Bitolterol, 699, 706
- Bivalirudin, 353
- Bleomycin, 527–528, 528f, 533, 610t, 612, 613f
- Blood, hemostasis of. *See* Hemostasis; Thrombosis
- Blood cells. *See* Hematopoiesis; *specific cells*
- Blood flow
  - abnormal, thrombosis in, 343, 343f
  - of gastric mucosa, 685
  - in organs, vs. distribution, 33, 33t
  - tissue distribution and, 35
- Blood pressure
  - abnormal. *See* Hypertension; Hypotension
  - determinants of, 318, 377, 377f
  - renin-angiotensin system antagonist effects on, 310–311, 311f
- Blood schizonts, in malarial parasite life cycle, 588, 588f
- Blood vessels, tone of. *See* Vascular tone
- Blood–brain barrier, 29, 84–86, 85f
  - impermeability of, to dopamine, 168
  - local anesthetic penetration of, 132
  - metabolic, 86
  - protein therapeutics crossing, 760
  - resistance to toxins, 721
  - toxins crossing, 726–727
- Blood/gas partition coefficients, of general anesthetics, 214–215, 214f, 215f, 219, 219f, 220
- Body water
  - compartments of, 300
  - regulation of. *See* Volume regulation
- Bonding. *See* Binding
- Bone(s), 473–487
  - disorders of. *See* Bone disorders
  - growth of, glucocorticoid effects on, 432
  - physiology of, 474–479
    - hormonal control, 476–479, 477t, 478f
    - mineral balance, 475, 475f
    - remodeling, 475–476, 476f
    - structure, 474–475, 474f
  - SERM effects on, 450–451, 451f, 450t
- Bone disorders, 479–483, 480t
  - in chronic renal failure, 480t, 482–483
  - drugs for, 483–489
    - affecting plasma calcium concentrations, 486–487, 489
    - anabolic agents, 485–486, 489
    - antiresorptive agents, 483–485, 484f, 485f, 488
    - oral calcium, 486, 489
    - vitamin D, 486, 489
  - in familial hypocalciuric hypercalcemia, 480t, 481
  - in hyperparathyroidism, 312, 432, 480–481, 480t
  - in hypoparathyroidism, 480t, 481
  - osteomalacia, 479–480
  - osteoporosis. *See* Osteoporosis
  - Paget's disease, 480t, 482
  - in pseudohypoparathyroidism, 480t, 481
  - rickets, 479–480, 480t
- Bone mineral density, in osteoporosis, 481–482, 482f
- Bortezomib, 613
- Botulinum toxin, 104
  - action of, 91f, 98
  - toxicity of, 732t
- Brachial plexus blocks, 132
- Bradycardia, in conduction block, 273
- Bradykinins
  - in nociception, 122, 123f
  - receptor for, in pain physiology, 232
  - angiotensin-converting enzyme inhibitor effects on, 310, 310f
  - in vascular tone regulation, 320, 321f
- Brain
  - anatomy of, 75–77, 75f–77f
  - blood–brain barrier and. *See* Blood–brain barrier
  - ischemia of, glutamate receptors in, 144–145, 145f, 146f
  - in pain transmission pathway, 123, 126f
- Brain partial pressure, of anesthetics, 213
- Brain-derived neurotrophic factor (BDNF), in pain transmission, 232, 236, 236f
- Brainstem
  - anatomy of, 75f, 77
  - dopamine receptors in, 164, 165f
  - in pain transmission pathway, 123, 126f
- Brand name, 798
- Breast
  - carcinoma of, estrogen receptor in, 448
  - SERM effects on, 450–451, 451f, 450t
- Bretylium, 280
- British anti-lewisite (dimercaprol, BAL), 741, 741f
- Brodifacoum, toxicity of, 732t, 742–743
- Brofaromine, 114, 118, 119, 188

Bromocriptine
  for Parkinson's disease, 169, 176
  for prolactinoma, 408, 413
Bronchial smooth muscle, histamine effects on, 648, 648t
Bronchoconstriction, in asthma, 696–699, 697f, 698f
Bronchodilators, for asthma, 699–700, 699f, 706
B-type natriuretic peptide
  for congestive heart failure, 311, 315
  in volume regulation, 303, 303f
Buccal preparations, of nitroglycerin, 324
BuChE (butyrylcholinesterase, pseudocholinesterase), 93
Budesonide, 701, 707
Buffering capacity, vs. toxic damage, 722
Bumetanide
  for congestive heart failure, 395–396
  for volume regulation, 312, 316
Bundle of His, 270–271
Bundle of Kent, 274, 274f
Bupivacaine, 134, 135
  for central nerve blockade, 132
  volume of distribution of, 131
Buprenorphine, 239, 244, 261, 263
Bupropion, 189, 191
Burimamide, 652
Burns
  chemical, macromolecular damage in, 722
  pain treatment in, 229–230
Burst firing, in GABAergic transmission, 148
Butorphanol, 239, 244
Butyrates, for sickle cell disease, 662
Butyrophenones, 171, 177
Butyrylcholinesterase (BuChE, pseudocholinesterase), 93

**C**

C cells, parafollicular, 415
C peptide, 459, 461f
Cabergoline, for prolactinoma, 408, 413
CAD. *See* Coronary artery disease (CAD)
Cadmium, toxicity of, 727, 732t
Caffeine, 259
  abuse of, 248t
  action of, 83
Calcifediol, 478, 478f, 489
Calcineurin, inhibitors of, 674
Calcitonin, 415, 485, 488
  in calcium homeostasis, 475, 475f, 477t, 479
  for hypercalcemia, 487
Calcitonin gene-related peptide (CGRP), in pain transmission, 123f, 232, 233f
Calcitriol, 475, 475f, 478–479, 478f, 489
Calcium
  deficiency of, treatment of, 487
  excess of. *See* Hypercalcemia
  homeostasis of, 475, 475f, 476–479, 477t, 478f
  importance of, 476
Calcium carbonate, 486, 489, 690, 693
Calcium channel blockers (CCBs)
  action of, 323f
  for arrhythmias, 281, 281f
  chemical classes of, 327, 328f, 328t
  contraindications to, 328
  for coronary artery disease, 387, 388
  for hypertension, 381
  mechanism of action of, 327
  N-type, for pain, 232
  pharmacokinetics of, 327–328
  toxicity of, 328
  for vascular tone regulation, 322, 327–328, 328f, 328t, 332
Calcium current, in cardiac action potential, 268, 269f, 270, 270t, 271f
Calcium disodium edetate (EDTA), 741, 741f
Calcium gluconate, for calcium abnormalities, 486, 489
Calcium hydroxide, toxicity of, 732t
Calcium ion
  in electrochemical transmission, 66–67, 66f
  in gastric acid secretion, 684, 685f
  Nernst equilibrium potential for, 62, 62t
  reabsorption of, thiazides for, 313
Calcium ion channel(s)
  in acetylcholine synthesis, 91f
  in afterdepolarization, 272–273, 273f
  in AMPA receptor activation, 142, 143t
  blockers of. *See* Calcium channel blockers (CCBs)
  in cardiac contractility, 286–289, 289t, 290f, 291, 293, 293f
  in cardiac function, 268, 269f, 270, 270t, 271f
  in catecholamine release, 109
  digoxin effects on, 293, 293f
  in dopamine release, 164
  in GABA receptor activation, 148–149, 148f
  in glutamate receptor activation, 142–143, 143f, 144t
  L-type, voltage-gated, in vascular tone regulation, 319, 319f, 320f
  neurotransmitter effects on, 140, 140f
  N-type, voltage-gated, in pain transmission, 232, 233f
  in peripheral sensitization, 235, 235f
  pharmacology of, 66
  selective, in vascular tone regulation, 318–319, 319f
  T-type
    in absence seizure, 197, 199f
    antiepileptic drug effects on, 201, 203
  in vascular tone regulation, 320, 321f
Calcium pump, in cardiac contractility, 287–288, 290f
Calcium sensitizing drugs, inotropic effects of, 297
*Calor,* as classic sign of inflammation, 617
cAMP. *See* Cyclic adenosine monophosphate (cAMP)
Campath-1, for immunosuppression, 677, 681
Camptothecins, 522f, 529, 533, 610t
Canada, drug approval in, 798
Canadian Food and Drugs Act, 798
Cancer
  cellular drug targets in, 495, 499, 500f, 501f
  chemotherapy for. *See* Antineoplastic drugs
  gene therapy for, 763t, 764, 770, 770f
  inflammation in, 638
  log cell kill model of, 499–500, 501f, 609
  origin of (carcinogenesis), 498–499
  pathophysiology of, 609
  proliferation in, 498–499, 609
  prostate, hormone treatment for, 448
  refractory or recurrent, treatment of, 612–613
  smoking-related, 729
  testicular, 519–520, 612, 613f
  vaccines for, 768
Candesartan, 315
Candidiasis, oropharyngeal, from glucocorticoid therapy, 433
Cannabinoid(s), 259
  abuse of, 248t
  receptors for, 233
Capacitor, in cell membrane, 60, 60f
Capacity, in tissue distribution, 35
Capillary permeability, volume regulation and, 300, 301f
Capsid, 546, 554
Captopril
  for vascular tone regulation, 333
  for volume regulation, 310, 315
Carbachol, 104
  action of, 101, 101t
  structure of, 100f
Carbamazepine
  action of, 200f
  for bipolar disorder, 185, 192
  for pain, 241, 245
  for seizures, 201, 203
Carbapenems, 568f, 572f, 575, 578, 573
Carbaryl, toxicity of, 724
Carbenicillin, 573, 575, 577
Carbidopa
  action of, 86
  levodopa with, for Parkinson's disease, 168, 168f
  for Parkinson's disease, 176
Carbon monoxide, poisoning from, 724, 726f, 732t, 742
Carbon tetrachloride, toxicity of, 722, 732t
Carbonic anhydrase II (CAII), in volume regulation, 305, 305f
Carbonic anhydrase inhibitors, for volume regulation, 311–312, 315
Carbonic anhydrase IV (CAIV), in volume regulation, 305, 305f
Carbonyl reduction, in drug metabolism, 47t
Carboplatin, 527, 527f, 533
Carboprost, 645
Carboxyhemoglobin, 726f
Carcinogen, definition of, 727
Carcinogenesis, 498–499, 609
  of cigarette smoke, 729
  from toxic exposures, 722, 727–728, 727f
Carcinoma, breast, estrogen receptor in, 448
Cardiac arrest, in anesthesia, 206t
Cardiac arrhythmia(s), 271–274
  case study of, 267–268
  drugs for. *See* Antiarrhythmic agents
  heart failure in, 392t
  in impulse conduction defects, 273–274, 274f
  in impulse formation defects, 272–273, 273f
  normal physiology and, 268–271, 269f, 270t, 271b, 271t, 272t
  pathophysiology of, 271–274, 273f, 274f
Cardiac contractility, 285–298
  β-adrenergic receptor antagonist effects on, 387–388
  calcium channel blocker effects on, 327, 328t
  as cardiac output determinant, 393, 393f
  dysfunctional. *See also* Congestive heart failure
    case study of, 285–286
    diseases causing, 289–291
    drugs for, 291–298
    inotropic agents for. *See* Inotropic agents
    pathophysiology of, 289–291, 292f, 293t, 391–393, 392f, 393f
  in myocardial oxygen demand, 318
  pathophysiology of, 289–291, 292f, 293t
  physiology of, 286–289
    myocyte anatomy in, 286, 287f, 288f, 289t
    myocyte contraction in, 286–287, 288f
    regulation of, 287–289, 289t, 290f, 291t
Cardiac fibrosis, aldosterone-mediated, 436
Cardiac glycosides, 291, 293–295, 293f, 294t, 298, 398
Cardiac output
  vs. anesthetic uptake, 211f, 212, 212f, 216–217, 216f
  as blood pressure determinant, 318
  calcium channel blocker effects on, 381
  in congestive heart failure, 394f

  - determinants of, 393, 393f
  - increased, hypertension in, 378
- Cardiac tamponade, heart failure in, 392t
- Cardiomyopathy, 391
- Cardiovascular effects
  - of histamine, 648t, 649
  - of lithium, 189
  - of local anesthetics, 132
- Carmustine (BCNU), 525f, 526, 533, 786
- Carvedilol, 116, 117t, 119, 279
- Caspofungin, 584
- Catalase, parasites lacking, 596
- Catalase peroxide, mutations of, 575
- Catatonic behavior, in schizophrenia, 170
- Catecholamine(s), 81–82, 82t, 83f, 84f, 107–119. *See also* Dopamine (DA); Epinephrine (EPI); Norepinephrine (NE)
  - actions of, 162, 183
  - in automaticity regulation, 272
  - case study of, 107–108
  - endogenous, physiologic effects of, 111–112
  - family members of, 162
  - metabolism of, 109–110, 109f, 110f
    - inhibitors of, 114, 118
  - physiologic effects of, 111–112
  - receptors for, 110–111, 111t
    - agonists of, 114–115, 118–119
    - antagonists of, 115–117, 119
  - release of, 108–109, 108f
  - reuptake of, 109–110, 109f, 110f
    - inhibitors of, 114, 118
  - storage of, 108–109, 108f, 109f
    - inhibitors of, 112–114, 113f, 118
  - structures of, 162, 162f
  - synthesis of, 108–109, 108f, 162–163, 162f
    - inhibitors of, 112, 118
  - in vascular tone regulation, 322
- Catechol-O-methyltransferase (COMT)
  - in catecholamine metabolism, 110, 110f
  - in dopamine degradation, 163, 164f
  - inhibitors of, for Parkinson's disease, 169
  - in monoamine degradation, 182
- Caudate, 75–76, 76f
  - dopamine receptors in, 164, 165f
- Caustic agents, macromolecular damage from, 722
- CBG (corticosteroid-binding globulin), 427, 431
- CCBs. *See* Calcium channel blockers (CCBs)
- CD3 monoclonal antibody (OKT3), for immunosuppression, 677, 681
- CD25 monoclonal antibody, for immunosuppression, 677, 681
- CD28, in costimulation, 677–678, 678f
- CD40 ligand, inhibitors of, 678–679, 678f
- CD52 monoclonal antibody, for immunosuppression, 677, 681
- Cefamandole, 575, 578
- Cefazolin, 575, 578
- Cefotaxime, 575, 578
- Cefotetan, 575, 578
- Ceftazidime, 575, 578
- Ceftizoxime, 575, 578
- Ceftriaxone, 575, 578
- Cefuroxime, 575, 578
- Celecoxib, 236, 240, 244, 641, 641f, 645
- Celiac ganglia, 72, 74f
- Celiprolol, 117, 117t, 119
- Cell(s), excitability of. *See* Excitability, cellular
- Cell adhesion blockade, 679, 681
- Cell cycle, antineoplastic drug interactions with, 499–500, 501f, 610t
- Cell (plasma) membrane
  - as barrier, 28–29, 29f
  - drug-receptor interactions and, 6–7
  - electrical circuit model of, 60, 60f
  - ion channels in, 7–8, 7f, 8t, 9f
  - receptors in
    - enzymatic cytosolic domains in, 7f, 9–12, 12f
    - G protein-coupled, 7f, 8–9, 10f, 11f, 11t, 68
- Cell wall
  - bacterial. *See* Bacterial cell wall synthesis
  - fungal, 580, 581f
- Cell-mediated immunity, 580, 622, 622f
- Cellular assays, in drug development, 753–754, 753t
- Cellular rejection, acute, in transplantation, 668–669, 668t
- Center-surround signaling, 78
- Central nerve blockade, 132
- Central nervous system. *See also* Brain; Spinal cord
  - affective disorders involving. *See* Affective disorders; Depression
  - anatomy of, 72, 75–78, 75f–77f
  - blood–brain barrier in. *See* Blood–brain barrier
  - general anesthetics affecting. *See* General anesthetics
  - local anesthetic effects on, 132
  - neurotransmission in
    - abnormal. *See* Seizure(s)
    - adrenergic, 180–183
    - dopaminergic, 161–178. *See also* Dopamine
    - GABAergic, 139, 147–159
    - glutamatergic, 139–147, 157
    - serotonergic, 180–183
  - pain transmission in, 123, 126f
- Central Pharmaceutical Affairs Council (Japan), 798
- Central sensitization, 145, 146f, 236, 236f
- Central tolerance, of immune system, 669
- Centrilobular necrosis, of liver, 726
- Cephalexin, 575, 578
- Cephalic phase, of gastric acid secretion, 684
- Cephalosporins, 568f, 572f, 573, 575, 578
- Cerebellar hemispheres, 76, 77f
- Cerebellar vermis, 76, 77f
- Cerebellum, anatomy of, 75f, 76–77, 77f
- Cerebral cortex, 75, 75f
- Cerebral malaria, 588, 589
- Cerebrospinal fluid, absorption into, 29
- Cerebrum, anatomy of, 75, 75f, 76f
- Cerulide, toxicity of, 729
- Cervical ganglia, 72
- Cetirizine, 654
- CETP (cholesterol ester transfer protein), 362, 364f, 365t, 366
- C-fibers, in pain conduction, 122, 123, 125f, 125t, 232
- cGMP (cyclic guanosine 3′5′-monophosphate), in vascular tone regulation, 320, 320f
- cGMP (cyclic guanosine 3′5′-monophosphate) phosphodiesterase type V inhibitors, 326
- cGMP-dependent protein kinase, in vascular tone regulation, 320, 320f
- CGRP (calcitonin gene-related peptide), in pain transmission, 123f, 232, 233f
- Chagas' disease, drugs for, 596
- ChAT (choline acetyltransferase), in acetylcholine synthesis, 90, 91f
- Chelation, of heavy metals, 740–741, 741f
- Chemical activators, in pain, 122, 123f, 231
- Chemical adsorption, of poisons, 738
- Chemical antagonist, 21, 21f, 23
- Chemical burns, macromolecular damage in, 722
- Chemical characterization, in drug development, 753, 753t
- Chemical dependence. *See* Dependence
- Chemical development, drug, 753–756, 753t, 754t
- Chemical exposures. *See* Toxic exposures
- Chemical gradient, 61, 61f
- Chemical mediators, of inflammation, 622–624, 623t. *See also specific substances*
- Chemical properties, of drugs, 754
- Chemical reaction, in polymer-based delivery systems, 784–785, 784f, 785f
- Chemical stimuli, pain response to, 230–232, 231f, 231t
- Chemical structure, characterization of, 754, 754t
- Chemical synthesis, in drug development, 753–757, 755f
- Chemical transmission, 69
- Chemical warfare agents, inhalation of, 721
- Chemistry review, of new drugs, 795, 796, 797f
- Chemokine receptors, in HIV replication, 546, 548f, 549f
- Chemoprophylaxis, 503
- Chemotaxis, 623t, 624
- Chemotherapy
  - antifungal. *See* Antifungal drugs
  - antimicrobial. *See* Antimicrobial drugs
  - antineoplastic. *See* Antineoplastic drugs
  - antiparasitic, 587–602
  - antiviral. *See* Antiviral drugs
  - combination, 603–613
    - antimicrobial, 603–608, 604f, 604t, 605f
    - antineoplastic, 608–612, 610t, 613f
    - antiviral, 608
- Chest pain, in myocardial ischemia, 318
- CHF. *See* Congestive heart failure (CHF)
- Chitin, in fungal cell wall, 580
- Chloral hydrate, 155
- Chlorambucil, 524, 526, 533
- Chloramphenicol, 537f, 539t, 541–542, 544
  - gray baby syndrome from, 53
- Chlordiazepoxide, 158
  - clinical applications of, 154t
  - ethanol interactions with, 155
- Chloride ion, Nernst equilibrium potential for, 62, 62t
- Chloride ion channels
  - barbiturate effects on, 150, 151f
  - benzodiazepine interactions with, 153, 153f
  - of GABA$_A$ receptor, 147–148, 149f
  - neurotransmitter effects on, 140, 140f
  - potentiation of, in ivermectin action, 598
- Chlorine, toxicity of, 726
- 2-Chloroprocaine, 133, 135
- Chloroquine, 589f, 590–591, 591f, 600
- Chlorpheniramine, 652, 652f, 654
- Chlorpromazine
  - for schizophrenia, 171, 177
  - structure of, 173f
- Chlorpropamide, 470
- Chlorprothixene, 177
- Chlorpyrifos, toxicity of, 724, 732t
- Chlortetracycline, 540, 543
- Chlorthalidone, 316
- Cholecalciferol, in calcium homeostasis, 477, 478f
- Cholesterol
  - absorption of, inhibitors of, 368, 373
  - adrenocortical hormone synthesis from, 426, 427f
  - dietary, digestion and absorption of, 359, 361f
  - elevated (hypercholesterolemia), 364–365, 365t
    - case study of, 357–358
    - drugs for. *See* Cholesterol-lowering drugs

Cholesterol, elevated (hypercholesterolemia) (*contd.*)
    familial, 364–365, 365t
    in mixed hyperlipidemia, 366
    secondary, 366–367, 366t
  homeostasis of, 360, 363f
  in lipoproteins, 358t
  metabolism of, 360, 363f
  reproductive hormones synthesized from, 442, 443f
  reverse transport of, 363
  synthesis of, 359–360, 362f
    inhibitors of, 368–369, 369f, 373
  transport of. *See* Lipoprotein(s)
Cholesterol ester transfer protein (CETP), 362, 364f
  defects of, 365t, 366
Cholesterol-lowering drugs
  bile acid absorption inhibitors, 367–368, 373
  cholesterol absorption inhibitors, 368, 373
  cholesterol synthesis inhibitors, 368–369, 369f, 373
  fibrates, 369–370, 370f, 374
  National Cholesterol Education Program guidelines for, 367, 367t
  niacin, 370–371, 371f, 373
Cholesteryl esters, formation of, 359–360, 361f–363f
Cholestyramine, 368, 373
Choline, in acetylcholine synthesis, 90, 91f
Choline acetyltransferase (ChAT), in acetylcholine synthesis, 90, 91f
Cholinergic drugs, case study of, 89–90
Cholinergic neurons, 80f
Cholinergic receptors
  muscarinic, 90–92, 91f, 92t
    agonists of, 100–101, 100f, 101t, 104
    antagonists of, 173
  nicotinic, 90–93, 91f, 92t, 93f, 94f
    agonists of, 101, 102t, 105
Cholinergic transmission. *See also* Acetylcholine (ACh)
  autonomic effects of, 94–95, 97f, 98t
  central nervous system effects of, 95–96
  drugs acting on, 96–103
    acetylcholine release inhibitors, 98, 104
    acetylcholinesterase inhibitors, 98–100, 99f, 104
    receptor agonists, 100–101, 100f, 100t, 104–105
    receptor antagonists, 102–103, 105
  in helminths, 598
  muscarinic, 91–92, 91f, 92t
    agonists of, 100–101, 100f, 101t, 104
    antagonists of, 173
  in neuromuscular junction, 94, 95f–97f
  nicotinic, 91–93, 91f, 93f, 94f
    agonists of, 101, 102t, 105
  physiologic effects of, 94–96, 95f–97f, 98t
  receptors in, 91–92, 93f, 94f
  in reward pathway, 257, 257f
Cholinesterase, 52, 93
Chromosomal resistance, to antibiotics, 573
Chronic coronary artery disease, 384. *See also* Coronary artery disease (CAD)
Chronic rejection, in transplantation, 668t, 669
Chronic toxicity, 720
Chronotropy
  β-adrenergic receptor agonists and, 295
  positive, 289
Churg–Strauss syndrome, from leukotriene antagonists, 703
Chylomicrons and chylomicron remnants, 358t, 359, 361f
  in dysbetalipoproteinemia, 366
Cidal drugs, 604–605, 604f, 604t, 605f. *See also* Bactericidal drugs
  antifungal, 583, 604
Cidofovir, 551f, 554, 562
Cigarette smoking. *See* Smoking
Cilastatin, 575, 578
Cilostazol, 355
Cimetidine, 652, 653f, 654
  as cytochrome P450 inhibitor, 51, 52b
  development of, 749
  for peptic ulcer disease, 688, 688f, 693
Cinchona tree bark, 590
Cingulate gyrus, 76, 76f
Ciprofibrate, 370
Ciprofloxacin, 529, 534
Circulation, vascular tone in. *See* Vascular tone
Circumventricular organs, dopaminergic neurons in, 166
Cirrhosis
  edema in, 308–309, 309f
  from toxins, 726
Cisplatin, 527, 527f, 533, 612, 613f
Citalopram, 187, 191, 241
c-kit ligand (stem cell factor, steel factor), 657, 658
Cladribine, 509f, 514f, 515, 518
Clarithromycin, 541, 544, 691
Clavulanic acid (clavulanate), 572f, 573–575
CLC-K2 chloride channels, in volume regulation, 306
Clearance, 38–39, 38f, 39t, 41
Clindamycin, 542, 544
Clinical (primary) endpoints, in clinical trials, 796
Clinical hold, in preclinical evaluation, 795
Clinical trials, in drug design and development, 795–796, 795t
Clomiphene, 451, 455
Clomipramine, 191
Clonazepam, 157
  action of, 200f
  clinical applications of, 154, 154t
  for seizures, 202
Clonic phase, of seizure, 197
Clonidine, 119
  action of, 82
  for hypertension, 115, 381
  hypothalamic effects of, 76
  for pain, 233, 241
Clopidogrel, antiplatelet activity of, 347f, 348, 355
Clorazepate, 154t, 157
Clorgyline, 114, 118
Closed state, of sodium ion channels, 194, 195f
*Clostridium perfringens*, case study of, 565–566
Clot formation, in hemostasis
  abnormal. *See* Thrombosis
  normal, 336, 337f, 338–340, 341f, 342f
Cloxacillin, 573, 577
Clozapine, 174–175, 178
CNQX (AMPA receptor antagonist), 147
CNS. *See* Brain; Central nervous system; Spinal cord
CNS partial pressure ($P_{CNS}$), for anesthetics, 206–207, 213, 216–218
Coagulation cascade, 336, 338–340, 341f, 342f
Coagulation disorders
  hypercoagulability, 343–345, 343f, 344t. *See also* Thrombosis
  hypocoagulability, 344, 344b
Coagulation factors, 338–339, 341f, 342f
  heparin effects on, 349–350, 349f
  synthesis of, vitamin K in, 350–352, 351f
Coal dust
  pulmonary fibrosis from, 726
  toxicity of, 733t
Coating agents, for peptic ulcer disease, 690–691, 694
Cocaine, 118, 133, 135
  addiction to, 254, 254f, 257–259, 258f
  discovery of, 121
  mechanism of action of, 109f, 114, 257–259, 258f
  schizophrenia-like state due to, 171
  in TAC (tetracaine-adrenaline-cocaine), 131
Coccidioidomycosis, case study of, 579–580
Codeine (methylmorphine), 238, 244
Cognate receptor, loss of function in, gene therapy for, 762–763, 763t
Colchicine, 530f, 531, 534, 712, 713f, 715
Colesevelam, 368, 373
Colestipol, 368, 373
Colitis, pseudomembranous, 542
Collagen, in platelet adhesion, 336, 337f
Collagenase, therapy with, 777
Collecting duct, in volume regulation, 304f, 306–307, 307f
Collecting duct (potassium-sparing) diuretics, 313–314, 316, 380
Collectins, in phagocytosis, 624–625
Colloid, thyroid, 416, 417f
Colloidal bismuth, 690–691, 694
Combination therapy
  antifungal, 607
  antimicrobial. *See* Antimicrobial drugs, in combination therapy
  antineoplastic. *See* Antineoplastic drugs, in combination therapy
  for congestive heart failure, 398
  for contraception, 452–453
  for diabetes mellitus, 468, 469t
  for *Helicobacter pylori* infections, 691
  for HIV, 556b
Combinatorial chemistry, in drug design, 748, 751, 751t
Commercial manufacturing, 753–756
Common lymphoid stem cells, in immune response, 618, 619f
Compassionate use protocols, 797
Compensation, cardiac, in congestive heart failure, 393–395, 394f–396f
Competitive antagonists, 7, 21–22, 21f, 22f
Competitive substrate inhibition, 53
Complement, 623
Complex partial seizures (temporal lobe epilepsy), 196t
Composition of matter patents, 801
Compound-centered drug design, 748–749, 749f, 750t
COMT (catechol-O-methyltransferase)
  in catecholamine metabolism, 110, 110f
  in dopamine degradation, 163, 164f
  inhibitors of, for Parkinson's disease, 169
  in monoamine degradation, 182
Concentration effect, in general anesthesia, 219
Concentration-dependent killing, of drugs, 604, 604f, 604t, 605f
Concentric pattern, of hypertrophy, in congestive heart failure, 394
Conditioned tolerance, 251
Conductance, 60, 60f
Conduction block, in cardiac conduction, 273
Conduction system, of heart
  calcium channel blocker effects on, 327, 328t
  components of, 270–271
  defects of, 273–274, 274f
  physiology of, 268–270, 269f, 270t, 271b, 271f, 272f, 273, 274f
Congenital adrenal hyperplasia, 437–438, 437f
Congestive heart failure (CHF), 391–399
  acute, case study of, 299–300

  - from anthracyclines, 529
  - case study of, 285–286, 391
  - clinical management of, 395–399, 397*t*
    - afterload reduction in, 396–398
    - combination therapy in, 398
    - inotropic agents in, 398. *See also* Inotropic agents
    - loop diuretics in, 312
    - preload reduction in, 395–396
    - thiazide diuretics in, 313, 316
  - clinical manifestations of, 285, 290–291
  - decompensated
    - β-adrenergic receptor antagonist avoidance in, 388
    - calcium channel blocker avoidance in, 388
  - diastolic, 392–393, 392*f*
  - edema in, 307–308, 308*f*
  - epidemiology of, 391
  - pathophysiology of, 290, 291, 391–395, 392*t*
    - cardiac compensation, 393–395, 394*f*–396*f*
    - contractile dysfunction, 391–393, 392*f*, 393*f*
  - systolic, 391–392
- Conjugation
  - in drug metabolism, 35, 48*t*, 49, 51*f*
  - in drug resistance, 501
- Consumer Products Safety Commission (CPSC), 743
- Contact dermatitis, 724
- Contraceptives, 452–453
  - combination, 452–453
  - implantable, 784
  - male, 453
  - morning-after, 453
  - oral, 452–453
    - toxicity of, 726, 736*t*
  - progestin-only, 453
- Contractile state, of myocardium. *See* Cardiac contractility
- Contractility, cardiac. *See* Cardiac contractility
- Contraction, smooth muscle, in vascular tone regulation, 318–320, 319*f*, 320*f*
- Controllers, for asthma, 699
- Convergent signaling, 78*f*, 79
- Convergent synthesis, in combinatorial chemistry, 754–755, 755*f*
- Copper toxicity, 733*t*, 740–741, 741*f*
- Coronary artery disease (CAD)
  - chest pain in. *See* Angina
  - clinical management of, 387–389, 387*t*
  - contractile dysfunction in, 289
  - heart failure in, 391
  - nitrates for, 322–326, 324*f*, 325*f*, 331
  - pathophysiology of, 384–386, 385*f*, 386*f*
  - prevention of, statins in, 368–369
- Coronary flow, reduction of, in CAD, 384–385, 385*f*, 386*f*
- Coronary flow reserve, 385, 386*f*
- Coronary steal phenomenon, 323
- Corpus callosum, 75, 76*f*, 196
- Corpus luteum, 445–446, 446*f*
- Cortical collecting duct, in volume regulation, 306–307, 307*f*
- Cortical spreading depression, in migraine, 237
- Corticosteroid-binding globulin (CBG), 427, 431
- Corticosteroids. *See also* Glucocorticoid(s); Mineralocorticoids; *specific substances*
  - GABA receptor binding site for, 148*f*
- Corticotrophs, 405*t*
- Corticotropin-releasing hormone (CRH), 84, 405*t*, 406*f*, 408, 409*f*, 428–429
- Cortisol (hydrocortisone), 406*f*, 439
  - administration routes for, 433–434, 434*f*
  - dosing of, 432–433
  - duration of action of, 431–432, 432*t*
  - in energy homeostasis, 458, 458*t*
  - metabolism of, 427, 428*f*
  - oral, 432
  - potency of, 430, 432*t*
  - replacement of, 432
  - structure of, 428*f*, 430, 431*f*
  - synthesis of, 426, 426*f*, 427*f*7
- Cortisone, 439
  - formation of, 427, 428*f*
  - structure of, 431*f*
- Costimulation, of immune response, 619
  - inhibitors of, 677–679, 678*f*, 681
- Costs, drug, regulation of, 800–801
- Cosyntropin, 408
- Cough, from angiotensin-converting enzyme inhibitors, 310
- Coumadin, toxicity of, 742–743
- Counter-regulatory hormones, 458–459, 458*t*, 460*f*. *See also specific hormones*
- Coupling, in thyroid hormone synthesis, 416, 417*f*
- Covalent bonding, 5, 5*t*
- Coxibs, 240. *See also specific drugs*
- COXs. *See* Cyclooxygenases (COXs)
- CPSC (Consumer Products Safety Commission), 743
- Craniosacral (parasympathetic) nervous system, anatomy of, 73, 73*f*, 74*f*
- "Crank" (methamphetamine HCl), 114
- Cravings, for drugs, 253
- Cretinism, 417
- CRH (corticotropin-releasing hormone), 84, 405*t*, 406*f*, 408, 409*f*, 428–429
- Crisis, hypertensive, 383
- Crohn's disease
  - inflammation in, 637
  - tumor necrosis factor α inhibitors for, 675–676
- Cromakalim, in vascular tone regulation, 329, 332
- Cromolyn, 701–702, 707
- Crosslinking, in bacterial cell wall synthesis, 567, 568*f*, 569, 570*f*
  - inhibitors of, 572–575, 572*f*, 574*f*, 577–578
- Crossover design, of clinical trials, 795
- Cryoglobulinemia, 670*t*
- Crystal forms, in drug development, 754
- "Crystal meth" (methamphetamine HCl), 114
- CTLA-4 immunoglobulin G, in costimulation inhibition, 677–678, 678*f*
- C-type natriuretic peptide, in volume regulation, 303, 303*f*
- Current. *See also specific ions*
  - in Ohm's law, 60, 60*f*
- Cushing's disease, 430
- Cushing's syndrome, 430
- Cushing's ulcer, 687
- Cutaneous flushing, from niacin, 371
- Cyanide poisoning, 724–725, 733*t*, 739, 739*f*, 742
- Cyclic adenosine 3′5′-monophosphate (cAMP)
  - in cardiac contractility, 288–289, 290*f*, 293*t*
  - in drug-receptor signaling, 9, 11*f*
  - in opioid tolerance, 253*f*
  - regulation of, 345, 348
- Cyclic endoperoxide
  - in platelet activation, 336
  - in prostaglandin synthesis, 345
- Cyclic guanosine 3′5′-monophosphate (cGMP), in vascular tone regulation, 320, 320*f*
- Cyclizine, 652*f*
- Cyclooxygenases (COXs), 239–240
  - actions of, 629, 629*t*
  - in arachidonic acid metabolism, 638–641, 639*f*, 641*f*
  - in cancer, 638
  - inhibitors of, 345, 346*f*, 347*f*, 348, 638–641, 639*f*, 641*f*, 644
  - isoforms of, 629, 629*t*
  - type 1, 629, 629*t*, 639
  - type 2, 629, 629*t*
    - aspirin effects on, 639
    - inhibitors of, 240, 640–641, 641*f*, 644
  - type 3, 629
- Cyclophosphamide, 524, 525*f*, 526, 526*f*, 527, 673, 680
- Cyclopropane
  - blood/gas partition coefficient of, 215*f*
  - oil/gas partition coefficient of, 210*f*
- Cycloserine, 568*f*, 571, 571*f*
- Cyclosporine, 748
  - for immunosuppression, 674, 674*f*, 680
  - tissue targeting of, 786–787
- Cyproterone, 449*f*, 452, 455
- CYPs. *See* Cytochrome P450 enzymes
- Cyst(s), *Entamoeba histolytica*, 594, 594*f*
- Cysteinyl leukotrienes, 632, 634*f*
  - inhibitors of, 702–703, 702*f*, 707
- Cystic fibrosis
  - gene therapy for, 762–763, 763*t*
  - pancreatic enzymes for, 759
- Cytarabine (cytosine arabinoside, AraC), 506*f*, 510*f*, 515, 516*f*, 518
- Cytidine, structure of, 507*t*, 516*f*
- Cytidylate
  - in ribonucleotide synthesis, 508, 508*f*
  - structure of, 507*t*
- Cytochrome(s), of malarial parasites, 590, 590*f*
- Cytochrome *c* oxidase, cyanide binding to, 724–725
- Cytochrome P450 enzymes, 426
  - in drug metabolism, 35, 39, 39*t*, 47–49, 50*f*
    - aging and, 53
    - dietary effects on, 53
    - disease effects on, 53–54
    - drug interactions with, 53
    - vs. drug type, 46*t*
    - environmental effects on, 53
    - genetic differences in, 52
    - induction and inhibition of, 49, 51, 51*t*, 52*b*
    - nomenclature of, 48
    - phase I reactions, 35, 45–49, 46*t*–47*t*, 50*f*
    - substrate specificity of, 48
  - in ergosterol synthesis, 580
  - induction of, in toxic exposures, 738–739
  - polymorphisms in, 806–808, 807*t*, 808*f*
  - in proton pump metabolism, 689, 690*b*
- Cytokine(s), 624. *See also specific cytokines*
  - in arachidonic acid synthesis, 628
  - in asthma, 697–698, 697*f*
  - cortisol effects on, 428, 429*f*
  - inhibitors of
    - for immunosuppression, 674–676, 675*f*, 676*f*, 681
    - for inflammation, 641, 645
  - in pain physiology, 232
  - production of, T cells in, 622, 622*f*
  - release of, glucocorticoid inhibition of, 432
- Cytokine release syndrome, from anti-thymocyte globulin, 677
- "Cytokine storm," from amphotericin B, 583
- Cytomegalovirus, drugs for, 551*f*, 553–554, 560
- Cytoplasmic membrane, of bacterial cell wall, 566*f*
- Cytoprotection, prostaglandins in, 631, 631*t*
- Cytosine, 506, 507*t*, 520, 520*f*, 521*f*
- Cytosine arabinoside (cytarabine, AraC), 506*f*, 510*f*, 515, 516*f*, 518

Cytosine deaminase, 513, 583, 584*f*
Cytosine-specific permeases, in fungal membranes, 581*f*, 583, 585
Cytotoxic agents. *See also* Antineoplastic drugs
  for immunosuppression, 671–673, 671*f*–673*f*, 672*t*, 680
Cytotoxic T cells, 620*t*, 622, 622*f*

D

D cells, in gastric acid secretion regulation, 684
D2 hypothesis, of antipsychotic action, 174, 177–178
D4 hypothesis, of antipsychotic action, 174
DA. *See* Dopamine (DA)
Dacarbazine, 526
Daclizumab, 677, 681, 778
Dalfopristin, 542, 544
Dander, hypersensitivity to, 723, 736*t*
Dantrolene, for malignant hyperthermia, 220
DAP (diaminopimelic acid), in murein synthesis, 567, 568*f*
Dapsone, 511, 517
Darbepoetin, 661–662, 665, 776
Daunorubicin, 528*f*, 533, 786
Davis model, of bactericidal activity, 539, 540*f*
Dazoxiben, 642, 646
D1–D5 receptors, dopamine, 164, 165*f*
ddC (zalcitabine), 551*f*, 563
ddI (didanosine), 563
De novo pathway
  for purine synthesis, 710, 710*f*
  for viral genome replication, 546
"Dead-end" complex, in beta-lactam action, 570*f*, 572–573
Dealkylation, in drug metabolism, 46*t*
Deamination, in drug metabolism, 46*t*
Debrisoquin metabolism, polymorphism in, 807–808, 808*f*
Decarboxylation, in drug metabolism, 46*t*
Declaration of Helsinki, 793
Decoding, in translation, 537*f*, 538
Deep vein thrombosis
  heparin for, 350
  from oral contraceptives, 453
Deferoxamine, 741, 741*f*
Degenerin epithelial sodium channels (ENaC), 231–232, 306–307, 307*f*, 313
Dehalogenation, in drug metabolism, 47*t*
Dehydroepiandrosterone (DHEA), 437–438, 439, 443*f*
Dehydrogenation, in drug metabolism, 46*t*
Deiodination, of thyroid hormones, 416, 416*f*, 417, 417*f*
Delavirdine, 555–556, 557*f*, 564
Delayed afterdepolarization, in cardiac cells, 273, 273*f*
Delayed rectifier potassium channels, 65, 65*f*
Delayed-type hypersensitivity, 723*f*, 724
Delivery, drug, 781–787
  asthma, 703–704
  gaseous anesthetics, 216, 216*f*
  insulin, 781–782, 785–786
  intelligent, 785–786
  liposome-based, 782, 786–787
  oral, 782
  polymer-based systems for, 783–786, 784*f*, 785*f*
  pulmonary, 782–783
  targeted, 786
  transdermal, 783
Delta cells, somatostatin secretion from, 458
Delta opioid receptor, 233
Delusions, in schizophrenia, 170
Demeclocycline, 311, 410, 540, 543
Dendritic cells, 620, 620*t*
  in asthma, 697, 697*f*
  function of, 659
Denileukin diftitox, 797
Dental procedures, procaine for, 133
Deoxyadenosine, structure of, 507*t*, 551*f*
Deoxyadenylate, structure of, 507*t*
Deoxycytidine, structure of, 507*t*, 551*f*
Deoxycytidine monophosphate, 508, 508*f*
Deoxycytidylate, structure of, 507*t*
Deoxyguanosine, structure of, 507*t*, 551*f*
Deoxyguanylate, structure of, 507*t*
Deoxyribonuclease I, 759
Deoxyribonucleosides, structures of, 507*t*
Deoxyribonucleotides, structures of, 507*t*
Deoxythymidine, structure of, 507*t*, 551*f*
Deoxythymidylate, structure of, 507*t*
Deoxyuridine, structure of, 507*t*
Deoxyuridylate, structure of, 507*t*
Dependence
  on barbiturates, 153
  on benzodiazepines, 155
  definition of, 249–250
  diagnostic criteria for, 250*b*
  on drugs, list of, 248*t*
  mechanisms of, 251–253
  physical (physiologic)
    definition of, 249–250
    mechanisms of, 251
    on opioids, 238
  psychologic, 250–253
  treatment of, 259–263, 260*f*, 261*b*
Dephosphorylase, in murein polymerization, 567, 568*f*
Depolarization
  in action potential, 64*f*, 65
  in cardiac cells, 268, 269*f*, 270*t*
    abnormal, 272–273, 273*f*
    atrial, P wave representing, 271*b*, 272*f*
    sodium ion channel blocker effects on, 275, 276*f*, 277*f*
    ventricular, QRS complex representing, 271*b*, 272*f*
  of cell membrane, 60
  in cholinergic transmission, 94
  in local anesthetic action, 130*f*
  in seizures, partial, 195
Depolarizing neuromuscular blockade, 101
Depot glucocorticoids, 434
Depression
  in bipolar disorder, 184*b*, 185
  case study of, 180
  diagnostic criteria for, 184
  drugs for, 191–192. *See also* Antidepressants
  monoamine theory of, 184–185, 186*f*
  neurotransmission and, 180–183, 181*f*, 182*f*, 183*t*
  pathophysiology of, 183–185, 183*b*, 184*b*
Deracoxib, 641
Dermatitis, contact, 724
Dermatomal distribution, of spinal nerves, 75
Descriptive toxicology, 743
Desensitization
  of acetylcholine receptor, 93
  of cells, 14, 15*t*
  of monoamine receptors, 185, 186*f*
Desflurane, 227
  blood/gas partition coefficient of, 214*f*, 215*f*, 220
  perfusion-limited, 214
  pharmacology of, 220
  properties of, 208*t*
  structure of, 222*f*
  tissue/blood partition coefficients of, 213*t*
Design, drug
  compound-centered, 748–749, 749*f*, 750*t*
  macromolecules, 752–743
  structure-based, 751–752
  target-centered, 749–752, 751*f*
Desipramine, 191
  for drug addiction, 262
  for pain, 241, 245
Desmopressin, 313, 410
Desogestrel, 452, 456
Desulfuration, in drug metabolism, 46*t*
Detergents, macromolecular damage from, 722
Detoxication, 720–722, 738–739, 739*f*, 740*f*
Detoxification, for drug addiction, 259–260, 260*f*
Development, drug, 753–760
  biological characterization in, 757–758, 758*t*
  chemical characterization in, 754, 754*t*
  chemical synthesis in, 754–755
  formulation in, 753, 755–756, 756*t*
Dexamethasone, 439
  for fetal lung maturity, 434
  potency of, 430, 432*t*
  structure of, 430, 431*f*
Dexrazoxane, 529
Dextromethorphan, 236, 241, 245
DHEA (dehydroepiandrosterone), 437–439
DHFR. *See* Dihydrofolate reductase (DHFR)
Diabetes insipidus, 410, 411*f*
  nephrogenic, lithium-induced, 189
Diabetes mellitus
  case study of, 457–458
  complications of, 464–465, 764
  control evaluation in, 464–465
  drugs for, 465–468, 470–471
    combination therapy, 468, 469*t*
    glucose absorption inhibitors, 460*f*, 465–466, 470
    insulin replacement, 466–467, 466*t*, 470
    insulin secretagogues, 467, 470
    insulin sensitizers, 467–468, 471
    strategies for, 465
  gene therapy for, 763*t*, 764
  from glucocorticoid therapy, 432–433
  hypertension in, ACE inhibitors for, 382
  morbidity and mortality in, 464–465
  pathophysiology of, 462–465, 463*t*
  type I, 463–464, 463*t*, 670*t*
  type II, 464, 463*t*, 763*t*, 764
Diabetic ketoacidosis, 463
Diacylglycerol
  in drug-receptor signaling, 9, 11*f*
  in platelet activation, 339*f*
  in platelet aggregation, 338, 339*f*
Diagnosis, proteins for, 761, 779
Diaminopimelic acid (DAP), in murein synthesis, 567, 568*f*
Diapedesis, of leukocytes, in inflammation, 624, 625*f*
Diastolic blood pressure, significance of, 376, 377*t*
Diastolic heart failure, 290, 392–393, 392*f*
Diastolic ventricular wall stress, in myocardial oxygen demand, 318, 319*f*
Diazepam, 157, 228
  clinical applications of, 154, 154*t*
  ethanol interactions with, 155
  with general anesthesia, 221
  for opioid addiction, 259
  for seizures, 202
Diazinon, toxicity of, 724, 733*t*
Diazoxide, for hyperinsulinemia, 460*f*, 461*f*, 468–469, 471
Diclofenac, 240, 244, 640
Dicloxacillin, 573, 577

- Dicumarol, 350, 351*f*
- Dicyclomine, 690, 694
- Didanosine (ddI), 563
- Diencephalon
  - anatomy of, 75*f*, 76
  - dopamine receptors in, 164, 165*f*
- Diet
  - drug interactions with, 71–72
  - drug metabolism and, 53
  - for dyslipidemia, 367
  - MAOI interactions with, 188
  - for peptic ulcer disease, 691
- Dietary Supplement Health and Education Act of 1994, 801
- Dietary supplements, regulation of, 801
- Diethyl ether, 205, 227
  - blood/gas partition coefficient of, 214*f*, 215*f*, 220
  - delivery of, ventilation effects on, 216, 216*f*
  - oil/gas partition coefficient of, 210*f*, 220
  - pharmacology of, 220
  - properties of, 208*t*
  - structure of, 222*f*
  - tissue/blood partition coefficients of, 213*t*
- Diethylcarbamazine, 599, 602
- Differential functional blockade, by local anesthetics, 127–128
- Diffuse (single-source divergent) neuronal organization, 78*f*, 79–80, 79*t*, 80*f*
- Diffusely projecting neurotransmitter systems, 180
- Diffusion
  - facilitated, 28, 85–86
  - Fick's law of, 29
  - membrane, 28–29, 29*f*
  - for polymer-based delivery systems, 783–784, 784*f*
  - rate of, of inhaled anesthetics, 209–210
- Diffusion hypoxia, in general anesthesia, 219
- α-Difluoromethylornithine (eflornithine), 596, 601
- Digitalis, 285
- Digitoxin, 291, 294–295, 298
- Digoxin, 291, 293–294, 293*f*, 294*t*, 298, 750*t*
  - for congestive heart failure, 397*t*, 398
  - drug–drug interactions, 294
  - for impaired contractility, 285
  - toxicity of, 733*t*, 741
- Digoxin immune fab, 741, 778
- Dihydrofolate, reduction of, 508
- Dihydrofolate reductase (DHFR), 508
  - as drug target, 494–495
  - inhibitors of, 510*f*, 511–513, 511*t*, 512*b*, 517, 607
- Dihydroorotate dehydrogenase
  - inhibitors of, 673, 673*f*
  - in malaria parasite metabolism, 590, 590*f*
- Dihydropteroate synthase
  - in folate synthesis, 511*f*
  - inhibitors of, 509–511, 510*f*, 511*f*, 517
- Dihydropyridines, in vascular tone regulation, 327
- Dihydrotachysterol, 486, 489
- Dihydrotestosterone (DHT), 442, 443*f*
- Dihydroxyphenylacetic acid, in dopamine metabolic pathway, 164*f*
- L-Dihydroxyphenylalanine (L-DOPA), catecholamine synthesis from, 81, 84*f*, 108, 108*f*, 162, 162*f*
- Diiodotyrosine (DIT), in thyroid hormone synthesis, 416, 417*f*
- Diisocyanates
  - hypersensitivity to, 723
  - toxicity of, 733*t*
- Diisopropyl fluorophosphate, 99, 99*f*, 104
- Dilated cardiomyopathy, 391
- Diltiazem
  - for arrhythmias, 281
  - for coronary artery disease, 388
  - for hypertension, 381
  - in vascular tone regulation, 327–328, 328*t*, 332
- Dimercaprol, 741, 741*f*
- Dioxins
  - ingestion of, 720
  - toxicity of, 724, 733*t*
- Diphacinone, toxicity of, 733*t*, 742–743
- Diphenhydramine, 651, 652*f*, 654
- Diploid luminal parasites, 595
- Dipyridamole, antiplatelet activity of, 347*f*, 348, 355
- Direct ligand-gated conductance, in nicotinic transmission, 92, 93*f*
- Direct pathway, nigrostriatal, 166, 167*f*
- Direct vasodilators, definition of, 322
- Discovery, drug, 748–753, 752*t*, 792*f*
  - case study of, 747–748, 748*f*
  - compound-centered design in, 748–749, 749*f*, 750*t*
  - definition of, 748
  - macromolecules, 752–753
  - structure-based design in, 751–752
  - target-centered design in, 748–752, 751*f*
  - timeline for, 749*f*
- Disopyramide, 277–278, 283
- Disorganized speech, in schizophrenia, 170
- Distal convoluted tubule, in volume regulation, 304*f*, 306, 306*f*
- Distribution, 33–35
  - four-compartment model of, 35, 38*f*
  - of general anesthetics, 207–208, 220–221, 220*f*
  - kinetics of, 34–35, 35*f*–37*f*
  - of local anesthetics, 131
  - vs. organ, 33, 33*t*
  - of toxins, 721
  - volume of, 33–34, 33*t*, 34*f*, 39
- Distribution phase, 34
- Disulfiram, 260–261
- DIT (diiodotyrosine), in thyroid hormone synthesis, 416, 417*f*
- Diuresis, saline, for hypercalcemia, 487
- Diuretics, 312–314, 316
  - action of, 16, 304–307, 304*f*–307*f*
  - collecting duct (potassium-sparing), 313–314, 316, 380
  - for congestive heart failure, 395–396, 396*f*, 397*t*
  - loop, 312–313, 316, 380
  - osmotic, 312, 316
  - thiazide, 306*f*, 313, 316, 379–380, 395–396
- Divergent signaling, 78–79, 78*f*
- DNA
  - damage of, topoisomerase inhibitors in, 522*f*, 528–530, 533–534
  - in gene therapy. *See* Gene therapy
  - purine and pyrimidine analogs in, 515, 516*f*, 518
  - repair of
    - in carcinogen exposure, 728
    - in toxicity, 725
  - replication of, 521, 522*f*
  - structure of, 520–521, 520*f*, 521*f*
    - modifiers of, 524–528, 525*f*–528*f*, 533
  - supercoiling of, 521, 522*f*
  - synthesis of, 506*f*, 508–509, 508*f*, 509*f*, 511*f*
  - in viral life cycle, 546, 548*f*
- DNA gyrase, 521
  - inhibitors of, 530
- DNA polymerase
  - activity of, 552
  - inhibitors of. *See* Antiviral drugs, genome replication inhibitors
- DNA topoisomerases
  - in DNA replication and coiling, 521, 522*f*
  - inhibitors of, 522*f*, 528–530, 533–534
  - in transcription, 536
- Dobutamine, 115, 119
  - for congestive heart failure, 397*t*, 398
  - inotropic effects of, 295*t*, 296, 298
- Docetaxel, 531, 534
- *Dolor*, as classic sign of inflammation, 617
- Domoic acid, seizures induced by, 194
- Donepezil, 104
  - action of, 83
  - for Alzheimer's disease, 100
- L-DOPA (L-dihydroxyphenylalanine), catecholamine synthesis from, 81, 84*f*, 108, 108*f*, 162, 162*f*, 749
- Dopa decarboxylase, 86
- Dopamine (DA), 80, 161–178
  - action of, 112
  - catecholamine synthesis from, 108*f*, 109
  - deficiency of, Parkinson's disease in. *See* Parkinson's disease
  - function of, 164, 166, 166*f*
  - in hypothalamic-pituitary-prolactin axis, 407–408
  - inactivation of, 163, 163*f*
  - increased and dysregulated levels of, in schizophrenia, 170–175, 170*b*, 172*f*, 173*f*
  - inotropic effects of, 295–296, 295*t*, 298
  - metabolism of, 163, 164*f*
    - inhibitors of, 169, 176
  - monoamine oxidase inhibitor effects on, 114
  - neurons containing, 161, 164, 166, 166*f*
  - in nigrostriatal pathways, 166, 167*f*
  - precursors of, 168–169, 168*f*, 176, 749
  - receptors for, 112, 164, 165*f*
    - agonists of, 169, 176
    - antagonists of, 171–175, 172*f*, 173*f*, 177–178
    - drug affinity for, 171, 172*f*
  - release of, 163, 163*f*
  - reuptake of, 163, 163*f*
  - storage of, 163, 163*f*
  - structure of, 83*f*
  - synthesis of, 81, 84*f*, 108, 108*f*, 161–163, 162*f*, 181*f*
- Dopamine autoreceptors, 164
- Dopamine hydroxylase, in dopamine synthesis, 162*f*, 163, 181*f*
- Dopamine (DA) hypothesis, of schizophrenia pathogenesis, 171
- Dopamine transporter, 182
  - blocking of, by stimulant drugs, 257, 258*f*
- Dopaminergic neurons, 80*f*
- Dornase alfa, 776
- Dorsal columns, of spinal cord, 75
- Dorsal horn, 77, 77*f*
  - pain transmission in, 123, 126*f*, 232, 233*f*
- Dorsal root(s), 73, 75, 77, 77*f*
- Dorsal root ganglia, 73, 75, 77, 77*f*
- Dose–response relationships and curves, 19–20, 19*f*, 20*f*
  - in clinical trials, 795
  - of general anesthetics, 207, 208*f*
- Dosing and doses
  - vs. bioavailability, 30, 30*f*
  - excessive (toxic), 40, 41*f*
  - goals of, 40, 41*f*
  - insufficient (subtherapeutic), 40, 41*f*
  - loading, 42
  - maintenance, 42–43, 42*f*
  - maximum tolerated, 795

- Dosing and doses (*contd.*)
  - median effective, 20, 20*f*, 25
  - median lethal. *See* Median lethal dose ($LD_{50}$)
  - median toxic, 20, 20*f*, 25
  - minimum lethal, 794
  - principles of, 40–42, 41*f*, 42*f*
  - therapeutic, 40–43, 41*f*, 42*f*, 43*t*
- Dotrecogin alfa, 775
- Double blind study, in clinical trials, 795
- Down-regulation, of receptors, 14, 15*t*
- Doxazosin, 116, 119, 333, 381
- Doxepine, 191
- Doxorubicin (Adriamycin), 529, 533, 786
- Doxycycline, 540–541, 543
- Doxylamine, 651, 654
- Droperidol, 177
  - with general anesthesia, 221
- Drug(s)
  - abuse of. *See also* Addiction; Dependence; Tolerance
    - commonly prescribed, 255–256, 256*f*
    - definition of, 249
    - nontherapeutic, 259
    - related to therapeutics, 256–259, 257*f*, 258*f*
  - definition of, 3
  - delivery of. *See* Administration routes; Delivery, drug
  - development of, 753–756, 753*t*–756*t*, 755*f*
  - discovery of. *See* Discovery, drug
  - misuse of, definition of, 249
  - new. *See* New drug evaluation
  - rational design of, 7
  - regulation of. *See* Regulation, drug
- Drug Price Competition and Patent Term Restoration Act of 1984, 793*t*, 797–798
- Drug receptors. *See* Receptor(s), drug
- Drug–drug interactions
  - additive, 605–606, 605*f*
  - antagonistic, 605–606, 605*f*
  - metabolism and, 53
  - plasma protein binding and, 34, 34*f*
  - synergistic, 605–606, 605*f*
- Drug-receptor binding curves, 18, 18*f*
- dsRNA, in gene therapy, 771
- d4T (stavudine), 551*f*, 563
- dUMP (deoxyuridylate), in nucleotide synthesis, 506, 508*f*
- Durham–Humphrey Amendment to Food, Drug and Cosmetic Act, 793*t*, 801
- Dust exposures, pulmonary fibrosis from, 726
- Dynamic instability, of microtubules, 523, 524*f*
- Dynorphins, 84, 233
- Dyskinesia, in Parkinson's disease, 169
- Dyslipidemia, 363–367, 365*t*
  - case study of, 357–358
  - in diabetes mellitus, 464
  - drugs for, 367–373
    - bile acid absorption inhibitors, 367–368, 373
    - cholesterol absorption inhibitors, 368, 373
    - cholesterol synthesis inhibitors, 368–369, 369*f*, 373
    - fibrates, 369–370, 370*f*, 373
    - National Cholesterol Education Program guidelines for, 367, 367*t*
    - niacin, 370–371, 371*f*, 373
  - genetic factors in, 365*t*
  - HDL metabolic disorders, 365*t*, 366
  - hypercholesterolemia, 364–365, 365*t*
  - hypertriglyceridemia, 365–366, 365*t*
  - mixed hyperlipidemia, 365*t*, 366
  - secondary hyperlipidemia, 366–367, 366*t*
- Dysrhythmia, cardiac. *See* Antiarrhythmic agents; Cardiac arrhythmia(s)

**E**

- Early afterdepolarization, in cardiac cells, 272–273, 273*f*
- Early-acting (general) growth factors, for hematopoiesis, 658
- $EC_{50}$ (potency), 40
  - of anesthetics, 223, 223*f*
  - of drug, 18, 18*f*
    - antagonist effects on, 22*f*
    - spare receptor effects on, 24–25, 24*f*
- Eccentric pattern, of hypertrophy, in congestive heart failure, 394
- Echinocandins, 583
- Ecstasy (methylenedioxymethamphetamine), 248*t*, 259
- Ectopic rhythm, in sinoatrial nodal dysfunction, 272
- $ED_{50}$ (median effective dose), 20, 20*f*
  - in therapeutic index, 25
- Edema
  - definition of, 307
  - histamine in, 649, 650*f*
  - loop diuretics for, 312
  - pathophysiology of, 307–309, 308*f*, 309*f*
  - transudative, 307–309
- Edinger–Westphal nucleus, 73
- Edrophonium, 104
  - action of, 99
  - clinical applications of, 100
  - structure of, 99, 99*f*
- EDTA (calcium disodium edetate), for heavy metal poisoning, 741, 741*f*
- EEG. *See* Electroencephalography (EEG)
- EET (epoxyeicosatetraenoic acid), 628*f*, 633
- Efalizumab, 681
- Efavirenz, 555–556, 557*f*, 564
- Effective refractory period, of myocytes, 276*b*
- Efficacy ($E_{max}$), of drug, 18, 18*f*, 23
- Efflux, drug, in drug resistance, 502
- Efflux pumps, in tetracycline resistance, 540
- Eflornithine, 596, 601
- Eggs, of helminths, 597
- Egress, in viral life cycle, 546, 547*f*, 548*f*
- Eicosanoids, 623–624. *See also* Arachidonic acid; *and specific metabolites*
  - in asthma, 637
  - in cancer, 637
  - definition of, 627
  - degradation of, 633
  - general scheme of, 633, 636, 636*f*, 636*t*
  - in glomerulonephritis, 637
  - in inflammatory bowel disease, 637
  - inhibitors of
    - cyclooxygenase, 638–641, 639*f*, 641*f*, 644
    - leukotriene, 642–643, 642*f*, 646
    - lipoxins, 643, 646
    - phospholipase, 638, 645
    - prostanoid receptor mimetics, 641, 645
    - thromboxane antagonists, 642, 646
  - in rheumatoid arthritis, 637
- Eicosatetraenoic acid. *See* Arachidonic acid
- EKG. *See* Electrocardiography (EKG)
- Elastase, 726
- Elastin, toxic destruction of, 726
- Elderly persons
  - antihypertensive agents for, 383
  - drug half-life in, 39
  - drug metabolism in, 53
- Electrical cycling, of heart. *See* Heart, electrical physiology of
- Electrocardiography (EKG), description of, 271*b*, 272*f*
- Electrochemical gradient, 61*f*, 62
- Electrochemical transmission, 66–69
  - definition of, 66
  - postsynaptic receptors in, 68
  - process of, 66–67, 66*f*
  - synaptic vesicle regulation in, 67–68, 68*f*
  - transmitter metabolism and reuptake in, 68–69
- Electroencephalography (EEG), in seizures
  - absence, 199*f*
  - partial, 195
- Electrogenic pumps, 62, 63*f*
- Electron transport chain, in malaria parasite metabolism, 590, 590*f*
  - inhibitors of, 590*f*, 592–593, 600
- Electrophiles, toxicity of, 722
- Electrophilic agents, DNA damage from, 520–521
- Electroporation, 783
- Electrostatic force, 61–62, 61*f*
- Elemental analysis, in drug development, 754
- Eletriptan, 245
- Elimination, of drugs
  - kinetics of, 34–35, 36*f*, 37*f*
  - of toxins, 721, 739–740
- Elimination half-life ($t_{1/2}$), 39, 39*t*, 40*b*, 43*t*
- Elongation stage
  - in transcription, 536, 536*f*
  - in translation, 538
- $E_{max}$ (efficacy), of drug, 18, 18*f*
  - antagonist effects on, 23
- Embolism, pulmonary
  - heparin for, 350
  - from oral contraceptives, 453
- Emergency, hypertensive, 383
- Emergency Use INDs, 794
- Emesis, in area postrema stimulation, 166
- EMLA (Eutectic Mixture of Local Anesthetic), 134, 135
- Emphysema, from smoking, 726
- ENaC (degenerin epithelial sodium channels), 231–232, 306–307, 307*f*, 313
- Enalapril
  - for vascular tone regulation, 333
  - in volume regulation, 310, 315
- Encainide, for arrhythmias, 278, 283
- Encephalopathy, toxic, 726–727
- "End of Phase II" meetings with FDA, 796
- End-diastolic volume, in cardiac contractility, 287
- Endocrine axes
  - definition of, 405
  - hypothalamic-pituitary-adrenal, 406*f*, 408, 409*f*
  - hypothalamic-pituitary-growth hormone, 406–407, 407*f*
  - hypothalamic-pituitary-prolactin, 407–408
  - hypothalamic-pituitary-reproductive, 408–410, 409*f*, 444–445, 444*f*, 445*f*, 447–448
  - hypothalamic-pituitary-thyroid, 408, 418, 419*f*
- Endocrine pancreatic hormones. *See* Diabetes mellitus; Glucagon; Insulin; Somatostatin
- Endocrine pharmacology. *See specific organs and hormones*
- Endocytosis, 28
- Endometriosis, 448
- Endometrium
  - in menstrual cycle, 446, 446*f*
  - oral contraceptive effects on, 452–453, 456
  - tamoxifen effects on, 450, 450*t*
- Endoplasmic reticulum, protein degradation in, 620–621, 621*f*
- β-Endorphin, in pain transmission regulation, 233, 429
- Endorphin(s), 84, 408
- Endothelial cells. *See* Endothelium, vascular
- Endothelial isoform of nitric oxide synthase, 320, 321*f*

Endothelial-derived relaxing factor (EDRF), 320. *See also* Nitric oxide (NO)
Endothelin
  in hemostasis, 336, 337*f*
  in vascular tone regulation, 321–322, 321*f*
Endothelin receptor antagonists
  action of, 323*f*
  inotropic effects of, 297
  in vascular tone regulation, 322
Endothelin-converting enzyme, 321, 321*f*
Endothelium, vascular
  dysfunction of, in CAD, 385–386
  histamine effects on, 648*t*, 649
  injury of, thrombosis in, 343, 343*f*
  in vascular tone regulation, 320–322, 321*f*
End-plate potential (EPP), 94, 96*f*
End-plate region, of muscle fibers, 95*f*
Endpoints
  clinical (primary), 796
  surrogate (secondary), 796
End-systolic pressure-volume relationship (ESPVR), in congestive heart failure, 393, 393*f*
Energy homeostasis, hormones in, 458–459, 458*t*, 460*f*
Enflurane, 227
  blood/gas partition coefficient of, 220
  oil/gas partition coefficient of, 210*f*, 220
  pharmacology of, 220
  properties of, 208*t*
  structure of, 222*f*
  tissue/blood partition coefficients of, 213*t*
  ventilation-limited, 214
Enfuvirtide (T-20), 547–548, 547*f*, 549*f*, 562
Enkephalins, 84, 233
Enolpyruvate transferase (MurA), 567, 568*f*
eNOS (endothelial isoform of nitric oxide synthase), 320, 321*f*
Enoxaparin, 350
Entacapone, 169, 176
*Entamoeba histolytica*, 593–597
  anti-protozoal agents for, 595–596, 601
  case study of, 593
  physiology of, 593–594, 594*f*, 595*f*
Enteral drug administration, 30–31, 31*t*, 755, 756*t*, 782
Enteric coatings, 756
*Enterobius vermicularis* (pinworms), drugs for, 598–599, 602
Enterochromaffin-like cells, histamine synthesis in, 647–648, 684
Enterococci, vancomycin-resistant, 572
Enterohepatic circulation, 38
Entricitabine (FTC), 563
Entry, in viral life cycle, 546, 547*f*, 548*f*
Envelope, viral, 546, 548*f*
Environmental factors
  in addiction, 254–255
  in diabetes mellitus, 464
  in drug metabolism, 53
Environmental toxicology, 719
Enzyme(s). *See also specific enzymes*
  activity of, evaluation of, in drug development, 753
  as drugs and drug targets, 13, 759, 773–777
  intracellular, loss of function in, gene therapy for, 762–763, 763*t*
  irreversible inactivation of, 53
  toxicity to, 724–725, 725*f*, 726*f*
Enzyme immunoassays, for HIV, 761, 779
Eosinophils, 619, 619*f*, 620*t*, 656*t*, 657*f*
  in asthma, 697*f*, 698, 698*f*
  function of, 659
  production of, 659
EPA (Environmental Protection Agency), 743
Ephedra, 801
Ephedrine, 113, 118
Epidermal thymocyte activating factor (ETAF), recombinant, 775
Epidural anesthesia, 132
Epilepsy
  AMPA receptors in, 145, 147
  case study of, 193–194
  vs. seizure, 193
  temporal lobe (complex partial seizures), 193–194, 196*t*
15-Epi-lipoxins, aspirin-triggered, 639
Epinephrine (EPI), 80, 162
  action of, 112
  for asthma, 699, 706
  in energy homeostasis, 458, 458*t*
  inotropic effects of, 295*t*, 296, 298
  with local anesthetics, 130–131
  receptor desensitization by, 14, 15*f*
  receptors for, 112
  for shock, 114
  structure of, 83*f*, 181*f*
  synthesis of, 82, 84*f*, 108–109, 108*f*, 162–163, 162*f*, 181*f*
  in TAC (tetracaine-adrenaline-cocaine), 131
  in vascular tone regulation, 322
Epipodophyllotoxins, 522*f*, 529, 610*t*
Epirubicin, 533
Eplerenone, 314, 316, 437, 439
Epoprostenol, 645
Epoxide formation, in drug metabolism, 46*t*
Epoxide reductase, in vitamin K action, 351, 351*f*
Epoxyeicosatetraenoic acid (EET), 628*f*, 633
Epoxygenase pathway, for arachidonic acid metabolism, 628*f*, 633
Epoxytetraene, 635*f*
Epstein-Barr virus
  as gene therapy vector, 766*t*, 769–770
  mycophenolate mofetil effects on, 673
Eptifibatide
  antiplatelet activity of, 348, 355
  for unstable angina, 389
Equations, for general anesthetic pharmacology, 226
Equilibration, of anesthetics. *See also* Uptake model
  global, 210
Equilibration time constant, of anesthetics, 212–215, 213*t*, 214*f*, 216, 226
Erectile dysfunction, sildenafil for, 326
EREs (estrogen response elements), 442, 444
Ergocalciferol, 477, 478*f*, 489
Ergosterol
  compounds binding with, 581*f*, 582–583, 585
  synthesis of, 580
    inhibitors of, 581–582, 581*f*, 585
Ergot derivatives, for Parkinson's disease, 169
Ergotamine tartrate, for migraine, 241
Erythrocytes, 656, 656*t*, 657*f*
  production of, 658–659, 659*f*, 660*t*
  sludging of, in malaria, 589
Erythrocytic stage, of malaria, 588, 588*f*
Erythromycin, 541, 544
Erythropoiesis, 658–659, 659*f*, 660*t*
  agents promoting, 662–662, 665
Erythropoietin, 749, 748*t*, 759
  action of, 657, 658–659, 659*f*, 660*t*
  therapy with, 774
Escape beats, in sinoatrial nodal dysfunction, 272
Escape rhythm, in sinoatrial nodal dysfunction, 272
Esmolol, 117, 117*t*, 119
  for arrhythmias, 283
  pharmacodynamics of, 387*t*
Esomeprazole, for peptic ulcer disease, 689, 693
ESPVR (end-systolic pressure-volume relationship), in congestive heart failure, 393, 393*f*
Estazolam, 154*t*, 158
Ester-linked local anesthetics, 124, 126*f*, 131, 131*f*, 133, 135
Estradiol
  potency of, 442
  structure of, 484*f*
  tissue targeting of, 786
Estramustine, 527, 533
Estrogen(s)
  actions of, 442, 444
  alkylating agent conjugated to, 527
  in calcium homeostasis, 479
  in contraceptives, 452–453, 456
  deficiency of, 448, 481–482, 482*f*
  inhibitors of, 449–450, 449*f*, 456
  in menstrual cycle, 445–446, 446*f*
  receptor for, 442, 444
    in breast carcinoma, 448
    selective modulators of. *See* Selective estrogen receptor modulators (SERMs)
  regulation of, 444*f*, 445, 445*f*
  release of, 409*f*
  replacement of, 453–454, 483–484, 488
  synthesis of, 442, 443*f*, 445, 445*f*
Estrogen response elements (EREs), 442, 444
Estrone
  after menopause, 448
  synthesis of, 443*f*
ET (endothelin), 321–322, 321*f*
ETAF (epidermal thymocyte activating factor), recombinant, 775
Etanercept, 641, 645, 675–676, 676*f*, 681, 776
Ethacrynic acid, in volume regulation, 312, 316
Ethambutol, 575, 578, 606
Ethanol. *See* Alcohol
Ethanolamines, 652*f*
Ether. *See* Diethyl ether
Ethics, of clinical drug investigation, 793–794
Ethinyl estradiol, 452, 456
Ethnic factors, in hypertension management, 383
Ethosuximide, 200*f*, 201, 203
Ethylene glycol, toxicity of, 733*t*
Ethylenediamines, 652*f*
Ethynodiol, 452, 456
Etodolac, 640
Etomidate, 221, 222*f*, 227
Etoposide (VP-16), 529, 533
Etoricoxib, 641
Eukaryotes, fungi as, 579
European Medicines Evaluation Agency, 798
European Union, drug approval by, 798
Eutectic Mixture of Local Anesthetic (EMLA), 134, 135
Exchange transfusion, for toxin elimination, 740
Excipients, 782
Excitability, cellular, 59–66
  action potential, 63–66, 64*f*, 65*f*
  definition of, 59
  Goldman equation, 62–63
  ion channels, 60–61, 60*f*, 66
  Nernst equation, 61–62, 61*f*, 62*t*, 63*f*
  Ohm's law, 60, 60*f*
  resting potential, 61–62, 61*f*, 62*t*, 63*f*
Excitation–contraction (EC) coupling, in myocytes, 286
Excitatory neurotransmitters. *See also* Glutamate
  action of, 140, 140*f*
Excitatory postsynaptic potentials (EPSPs), 67, 94–95, 97*f*

Excitement stage, of general anesthesia, 206*f*
Excitotoxicity
  description of, 143–144
  diseases associated with, 144–147, 145*f*, 146*f*
Excretable metabolites, definition of, 45
Excretion, 35–38
  biliary, 36–38, 37*f*
  kinetics of, 38–39, 38*f*
  of local anesthetics, 131, 131*f*
  renal, 36–38, 37*f*
Exemestane, 449*f*, 450, 455
Exercise, asthma precipitation in, 696–697
Exo-erythrocytic hepatic stage, of malaria, 588, 588*f*
Expedited Drug Approval Act of 1992, 793*t*
Experimental autoimmune encephalitis, 670*t*
Extended release formulations, 782
External advisory committees, for FDA approval, 796
Extractable lipids, in mycobacterial cell wall, 566*f*, 569
Extraction ratio, 39
Extrapyramidal effects, of antipsychotic drugs, 171, 172
Extrinsic pathway, for coagulation, 339, 341*f*
Extrinsic (acquired) resistance, to antibiotics, 573
Ezetimibe, 368, 373

**F**

Facial nerve, parasympathetic fibers in, 73, 74*f*
Facilitated diffusion, 28, 85–86
Facilitation, of acetylcholine action, 94
Factor(s), coagulation, 338–339, 341*f*, 342*f*
  heparin effects on, 349–350, 349*f*
  synthesis of, vitamin K in, 350–352, 351*f*
Factor V Leiden mutation, 344–345, 808
Factor VIIa, recombinant, 775
Factor VIII, therapy with, 773
Factor IX, therapy with, 773
Falcilysin, in heme metabolism, 589, 589*f*
Falcipain, in heme metabolism, 589, 589*f*
False neurotransmitters, 113
Famciclovir, 551*f*, 552–553, 562
Familial adenomatous polyposis, 641
Familial dyslipidemias
  apoAI deficiency, 365*t*
  apoB100 defects, 365, 365*t*
  combined hyperlipidemia, 365*t*, 366
  dysbetalipoproteinemia, 365*t*, 366
  hypercholesterolemia, 364–365, 365*t*
    gene therapy for, 763, 763*t*
  hypertriglyceridemia, 365–366, 365*t*
  hypoalphalipoproteinemia, 365*t*
Familial hypocalciuric hypercalcemia, 481
Famotidine, 652, 654, 688*f*, 689, 693
Farmer's lung, 724
FAS1 and FAS2 (fatty acid synthases), in mycobacterial cell wall synthesis, 569, 571*f*
"Fast acetylators," 52, 806–807, 807*f*
Fast dissociation hypothesis, of antipsychotic action, 174
Fast excitatory postsynaptic potential, 97*f*
Fast vs. slow acetylators, 52
Fasting state, glucose homeostasis in, 459
Fat, deposition of, from glucocorticoids, 433
Fat group, in uptake model, 210, 211*f*, 219
Fatty acid(s), absorption and transport of, 359, 361*f*, 362*f*
Fatty acid synthase 1 and 2, in mycobacterial cell wall synthesis, 569, 571*f*
FDA. *See* Food and Drug Administration (FDA)
Fed state, glucose homeostasis in, 459, 460*f*
Feedback systems
  in hypothalamic-pituitary interaction, 405–406, 405*t*, 406*f*
  in volume sensing, 300–301
Felodipine, for coronary artery disease, 388
Fenamates, as NSAIDs, 639*f*, 640, 644
Fenestrae, in blood–brain barrier, 85, 85*f*
Fenofibrate, 370, 373
Fentanyl, 239, 244
  as anesthetic adjunct, 228
  with general anesthesia, 221
Fenthion, toxicity of, 724, 734*t*
Fermentation enzymes, lack of, in protozoal metabolism, 594–595
Ferredoxins, in *Entamoeba histolytica* metabolism, 594–595, 595*f*
Ferriprotoporphyrin IX
  in heme metabolism, 589–590, 589*f*
  inhibitors of, 591
Fertility, protein-based drugs for, 774
Fetal alcohol syndrome, 729
Fetal hemoglobin, 658
  inducers of, 662, 665
Fetus
  teratogen effects on, 447*b*, 722, 728
  warfarin-related defects of, 352
Fever, chemical mediators in, 623*t*
Fexofenadine, 654
Fibrates, 369–370, 370*f*, 373
Fibrillation
  atrial, 276*b*, 293
  ventricular, 267, 276*b*
Fibrinogen
  in platelet activation, 339*f*
  in platelet aggregation, 338
Fibrinoid arteriolar necrosis, hypertensive crisis in, 383
Fibrinolysis, 337*f*, 342, 343*f*
Fibrosis
  cardiac, aldosterone-mediated, 436
  pulmonary, from smoking, 726
  in toxic exposures, 722, 726
Fick's law of diffusion, 29, 226
  for inhaled anesthetics, 209–210
Fight-or-flight response, 108, 112
Filariasis
  drugs for, 598–599, 602
  physiology of, 597–598, 597*f*
Filgrastim, 662–663, 665
Filtration, fluid, 300, 301*f*
Finasteride, 449, 449*f*, 455
Firing rate, of conduction system, 270–271
First generation H1 antagonists, 651, 652*f*, 654
First pain, 122, 125*f*
First-order kinetics, in clearance, 38
First-pass effect, 31, 47, 49*f*
Fish oil, dietary, 631, 632*f*
FK506 (tacrolimus), for immunosuppression, 674, 674*f*, 680
FK-binding proteins (FKBPs), tacrolimus binding to, 674, 674*f*, 680
FLAP (5-lipoxygenase activating protein), 632, 634*f*
  inhibitors of, 642, 646
Flecainide, for arrhythmias, 278, 283
Flocculonodular lobe, 76, 77*f*
Fluconazole, 581, 581*f*, 582
Flucytosine, 510*f*, 511*f*, 513, 517, 581*f*, 583, 584*f*, 585
  amphotericin B with, 607
  structure of, 513*f*
5-Fludarabine phosphate, 506*f*, 509*f*, 514*f*, 515, 518
Fludrocortisone, 436, 439
  potency of, 430
  structure of, 431*f*
Fluid filtration, 300, 301*f*
Fluid volume regulation. *See* Volume regulation
Flumazenil, 158
  action of, 153
  for benzodiazepine reversal, 221, 742
  GABA receptor binding site for, 148*f*
Flunisolide, 439
  for asthma, 701, 707
  inhaled, 433
  structure of, 434*f*
Flunitrazepam, 155
Fluoride, for bone disorders, 485, 489
Fluoroquinolones, 529–530, 534
5-Fluorouracil, 510*f*, 511*f*, 513*f*, 517
Fluoxetine, 180, 191, 241, 262
Fluphenazine
  pharmacokinetics of, 174
  for schizophrenia, 171, 177
  structure of, 173*f*
Flurazepam, 154, 154*t*, 158
Flurbiprofen, 640
Flushing, from niacin, 371
Flutamide, 449*f*, 452, 455, 610*t*
Fluticasone, 439
  for asthma, 701, 707
  inhaled, 433
  structure of, 434*f*
Flutter, atrial, 276*b*, 277
Fluvastatin, 369, 373
Fluvoxamine, 187, 191
Foam cells, 361, 362*f*, 363*f*
Focus, of seizure, 195–196, 197*f*
Folic acid/folate
  functions of, 511*f*
  metabolism of, 508, 510*f*, 511*f*
    inhibitors of, 509–513, 510*f*, 511*t*, 512*b*, 517
  structure of, 508, 510*f*
  synthesis of, 511*f*
Folinic acid
  for colorectal cancer, 513
  as rescue, 512
Follicle-stimulating hormone (FSH), 404, 408–410, 409*f*, 444–445, 444*f*, 445*f*
  in menstrual cycle, 445–446, 446*f*
  recombinant
    for ovulation induction, 759
    therapy with, 774
Follicular phase, of menstrual cycle, 445, 446*f*
Follicular thyroid cells, 415, 416, 417*f*
Follitropin, 414
Fomepizole, for alcohol intoxication, 738
Fomivirsen, 560, 564, 771
Food
  MAOI interactions with, 188
  toxins in, 720–721
Food, Drug and Cosmetic Act of 1938, 793*t*
Food and Drug Administration (FDA), 743, 791
  approval process of. *See* New drug evaluation, approval phase of
  drug regulation after approval, 798–801, 799*f*, 801*t*
  ethical code of, 793–794
  history of, 792–793, 793*t*
  IND review by, 794–795, 794*f*
  new drugs approved by, 747
Food poisoning, 729
Forensic toxicology, 719
Formestane, 449*f*, 450, 455
Formic acid, toxicity of, 727
Formoterol, for asthma, 700, 706
Formulation, drug, 753, 755–756, 756*t*, 782

Formylated methionine, in translation, 537
Foscarnet (phosphonoformic acid, PFA), 555, 557*f*, 563
Fosfomycin, 568*f*, 570–571, 577
Fosinopril, 315
Fosmidomycin, 568*f*, 570–571, 577
Fraction absorbed. *See* Bioavailability
Fractional inhibitory concentration, of drugs, 605–606
Fractional occupancy, of receptors, 18*f*
Frank–Starling law, in cardiac contractility, 287
  in CHF, 392–394, 393*f*, 394*f*
Free radicals, toxicity of, 722
Fresh frozen plasma, for poisoning, 743
Frontal cortex, dopamine receptors in, 164, 165*f*
Frontal lobe, 75, 76*f*
FSH (follicle-stimulating hormone), 404, 408–410, 409*f*, 444–445, 444*f*, 445*f*
  in menstrual cycle, 445–446, 446*f*
  recombinant, 759
FTC (entricitabine), 563
Functional residual capacity, inhaled anesthetics and, 210–211
Fungi and fungal infections
  biochemistry of, 579
  case study of, 579–580
  drugs for, 580–585
    ergosterol synthesis inhibitors, 581–582, 581*f*, 585
    ergosterol-binding compounds, 581*f*, 582–583, 585
    targets of, 498
    thymidylate synthase inhibitors, 583, 584*f*, 585
  pathophysiology of, 580
  types of, 579
Furosemide
  for congestive heart failure, 395–396
  for hypertension, 380
  for volume regulation, 312, 316
Fusion, in viral replication, 546, 547*f*, 548*f*
  inhibitors of, 547–548, 549*f*, 562

**G**

G cells, gastrin secretion from, 684, 685*f*
G protein(s)
  activation of, GABA receptors in, 147, 148, 148*f*
  inhibitory, in cardiac contractility, 291
G protein-coupled receptors, 7*f*, 8–9, 10*f*, 11*f*, 11*t*, 68, 180
  in cardiac contractility, 289, 290*f*
  in pain, 235
GABA. *See* Gamma-aminobutyric acid (GABA)
GABA receptor(s)
  in alcohol abuse, 255–256
  antagonists of, 149*t*, 158
  in benzodiazepine abuse, 255–256
  $\text{GABA}_\text{A}$, 147–148, 148*f*, 149*f*
    agonists of, 149*t*, 150
    antiepileptic drug effects on, 201–202, 204
    in benzodiazepine abuse, 255–256
    in general anesthetic action, 223
    modulation of, 150–153, 151*f*, 151*t*, 152*t*, 153*f*
  $\text{GABA}_\text{B}$, 147–149, 148*f*
  $\text{GABA}_\text{C}$, 149
  modulators of, 149*t*
  in opioid abuse, 255, 256*f*
  physiology of, 147–149, 148*f*, 149*f*
Gabaculine, 149, 149*t*
Gabapentin
  for pain, 241, 245
  for seizures, 201–202, 204
Galantamine, 100, 104
Gallbladder disease, from oral contraceptives, 453
Gametocytes, in malarial parasite life cycle, 588, 588*f*
Gamma-aminobutyric acid (GABA)
  action of, 140, 140*f*
  antagonists of, GABA receptor binding site for, 148*f*
  degradation of, 147
  metabolism of, modulators of, 149*t*
  in nematode neuromuscular activity, 598
  in neuron firing regulation, 195, 195*f*, 198*f*
  as neurotransmitter, 80
  in pain transmission regulation, 232, 234*f*
  receptors for. *See* GABA receptor(s)
  release of, modulators of, 149*t*
  structure of, 83*f*
  synthesis of, 141, 141*f*, 147
    modulators of, 149*t*
Gamma-aminobutyric acid (GABA) transaminase, in glutamate synthesis, 141, 141*f*
Ganciclovir, 551*f*, 553–554, 555*t*, 562, 786
Ganglia, autonomic
  anatomy of, 72, 73*f*, 74*f*
  cholinergic transmission effects on, 94–95, 97*f*, 98*t*
  nicotinic receptor agonist effects on, 101
Ganglionic blockers, for hypertension, 381
Gangrene, case study of, 565–566
Gaseous anesthetics. *See* Inhaled anesthetics
Gastric acid secretion
  H2 receptor antagonist effects on, 652, 653*f*, 654
  in *Helicobacter pylori* infections, 686, 686*f*
  histamine regulation of, 649
  inhibitors of, 688–690, 688*f*, 689*f*, 690*b*, 693
  physiology of, 684–685, 685*f*
Gastric mucosa, protective agents for, 690–691, 694
Gastric phase, of gastric acid secretion, 685
Gastrin, 84
  in gastric acid secretion, 684–685, 685*f*
  secretion of, in *Helicobacter pylori* infections, 686, 686*f*
Gastrinoma, peptic ulcer disease in, 687
Gastrointestinal system
  histamine effects on, 648*t*, 649
  toxin absorption in, 720–721
Gastropathy, NSAID-induced, 638
G-CSF (granulocyte colony-stimulating factor), recombinant, for neutropenia, 662–663, 665, 774
G-CSF (granulocyte-colony stimulating factor), in hematopoiesis, 658–660
Gemfibrozil, 370, 373
Gemtuzumab ozogamicin, 777
Gender differences, in drug metabolism, 53
Gene(s)
  expression of, inhibitors of, for immunosuppression, 670–671, 680
  rearrangements of, in adaptive immunity, 621
  transcription of, 536–537, 536*f*, 537*f*
    inhibitors of, 538, 544
  translation of, 537–538, 537*f*
    inhibitors of, 538–542, 539*t*, 540*f*, 543–544
Gene therapy, 762–771
  advantages of, 762
  antisense strategies in, 770–771, 770*f*
  for contractility dysfunction, 297
  safety of, 764–765
  targets of, 762–764, 763*t*
  viral vectors for, 765–771, 766*t*
    adeno-associated viruses, 766*t*, 769
    adenoviruses, 766*t*, 768–769
    Epstein-Barr virus, 766*t*, 769–770
    herpesviruses, 766*t*, 769–770
    retroviruses, 765–768, 766*t*, 767*f*, 768*f*
General anesthetics, 205–228
  abbreviations related to, 225
  adjuvant drugs with, 221
  analgesic index of, 207, 208*f*
  balanced, 221
  barbiturates as, 150, 151, 152*t*
  case study of, 205–206
  cerebral cortical effects of, 75
  discovery of, 205
  distribution of, 220–221, 220*f*
  drugs for, 227–228. *See also specific drugs*
  equations involved in, 226
  induction of
    age factors in, 216, 217*f*
    cardiac output and, 216–217, 216*f*
  inhaled. *See* Inhaled anesthetics
  ion channel effects of, 222–223, 223*f*
  mechanism of action of, 221–223, 222*f*
  Meyer–Overton rule for, 207, 209*b*, 210*f*, 222
  minimum alveolar concentration of, 206–207, 207*b*, 208*t*
  partition coefficients of, 207, 209*b*
  perfusion-limited, 214, 214*f*, 216, 216*f*
  pharmacodynamics of, 206–207, 206*f*, 207*b*, 208*t*
  pharmacokinetics of, 207–219
    physiologic concepts in, 209–210
    recovery, 218–219, 219*f*
    uptake model of, 210–218, 211*f*, 212*f*, 213*t*, 214*f*–217*f*, 213*t*, 218*t*
  pharmacology of, 220–221, 220*f*
  redistribution of, 219
  stages of anesthesia from, 206, 206*f*
  symbols related to, 225
  therapeutic index of, 207, 208*f*
  ventilation-limited, 214, 216, 216*f*
General (multilineage) growth factors, for hematopoiesis, 658
Generalized seizures, 195, 196*t*
  secondary, 196–197, 196*t*, 197*f*, 198*f*
Generator currents, in nociception, 122
Generic drugs, approval of, 797–798
Generic name, 798
Genetic factors
  in addiction, 254–255
  in aspirin-induced asthma, 703
  in diabetes mellitus, 463–464
  in drug metabolism, 52, 806–808, 807*f*, 807*t*, 808*f*
  in drug resistance, 501–503, 502*t*
  in hypercoagulability, 344, 344*b*, 344*t*
  in idiosyncratic effects, 808–809, 808*t*
  in inherited diseases, 762–763, 763*t*
  in Parkinson's disease, 167
  population variations, 803–804, 804*f*
  in target proteins and pathways, 805–806, 805*t*
  in warfarin action, 352
Genome
  human, variation in, 803–804, 804*f*
    enzymes of drug metabolism, 806–808, 807*f*, 807*t*, 808*f*
    idiosyncratic effects and, 808–809, 809*t*
    target proteins and pathways, 805–806, 805*t*
  viral, replication of, in viral life cycle, 546, 547*f*, 548*f*
Gentamicin, 539, 543
GH. *See* Growth hormone (GH)
Ghrelin, 405*t*, 406, 407*f*, 413
GHRH. *See* Growth hormone-releasing hormone (GHRH)

- Gigantism, 407
- GIP (glucose-dependent insulinotropic polypeptide), 460
- Glargine insulin, 466*t*, 467, 470
- Glatiramer acetate, 779
- Glaucoma
  - carbachol for, 101
  - carbonic anhydrase inhibitors for, 311–312
- Glial cells, glutamate synthesis and degradation in, 141, 141*f*
- Glibenclamide, 470
- Gliclazide, 470
- Glimepiride, 470
- Glipizide, 470
- Gliquidone, 470
- Global equilibration, of anesthetics, 210
- Globus pallidus, 75–76
  - in movement control, 166
- Glomerular filtration, sympathetic control of, 304
- Glomerulonephritis, inflammation in, 637
- Glossopharyngeal nerve, parasympathetic fibers in, 73, 74*f*
- Glucagon
  - action of, 462
  - cells secreting, 458
  - in energy homeostasis, 458–459, 458*t*, 460*f*
  - for hypoglycemia, 468, 471
  - recombinant, 775
  - secretion of, 458
- Glucagon-like peptide-1, 460
- Glucans, in fungal cell wall, 580
- β-Glucocerebrosidase, therapy with, 758, 773
- Glucocorticoid(s), 426, 426*f*
  - actions of, 427–428, 429*f*, 634*f*
  - adverse effects of, 670
  - analogues of, 430–434, 439
    - administration routes for, 433–434, 434*f*
    - dosing of, 432–433
    - duration of action of, 431–432, 432*t*
    - potency of, 430–431, 432*t*
    - for replacement therapy, 432
    - structures of, 430, 431*f*, 434*f*
  - for asthma, 701, 707
  - in calcium homeostasis, 477*t*, 479
  - depot, 434
  - disorders of
    - case study of, 425–426
    - drugs for, 430–435, 430*f*, 431*f*, 432*t*, 434*f*, 435*t*, 439
    - excess (Cushing's syndrome), 430
    - insufficiency (Addison's disease), 429–430, 433
    - pathophysiology of, 429–430
  - for gout, 712–713, 715
  - immunosuppressive effects of, 670–671, 680
  - for inflammation, 638, 641, 645
  - inhaled, 433–434, 434*f*
  - metabolism of, 427, 428*f*
  - physiology of, 426–429, 426*f*–429*f*
  - in pregnancy, 434
  - receptors for, 431
    - antagonists of, 435, 439
  - regulation of, 428–429
  - synthesis of, 426, 426*f*, 427*f*
    - inhibitors of, 427*f*, 434–435, 435*t*, 439
  - withdrawal from, 433
- Glucocorticoid response elements (GREs), 428
- Glucose
  - absorption of, inhibitors of, 460*f*, 465–466, 470
  - fermentation of, by *Entamoeba histolytica*, 594–595, 595*f*
  - glucocorticoid effects on, 432
  - homeostasis of
    - abnormal. *See* Diabetes mellitus; Hypoglycemia
    - hormones in, 458–459, 458*t*, 460*f*
  - transport across blood–brain barrier, 85–86, 85*f*
- Glucose transporters, in insulin secretion, 459, 461*f*, 462
- Glucose-dependent insulinotropic polypeptide (GIP), 460
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
  - polymorphism in, 808–809
  - primaquine toxicity in, 593
- α-Glucosidase inhibitors, for diabetes mellitus, 460*f*, 465–466, 470
- Glucuronidation, in drug metabolism, 48*t*, 49, 51*f*
- GluR1-GluR4 subunits, of AMPA receptor, 142, 143*t*
- GluR5-GluR7 subunits, of kainate receptor, 142, 143*t*
- GLUT transporters, in insulin secretion, 459, 461*f*, 462
- Glutamate
  - action of, 140, 140*f*
  - degradation of, 141, 141*f*
  - as neurotransmitter, 80
  - release of, inhibitors of, 157
  - structure of, 83*f*
  - synthesis of, 141, 141*f*, 147
- Glutamate receptor(s)
  - classes of, 140
  - interactions among, 146*f*
  - ionotropic, 141–142, 142*f*, 143*t*
  - metabotropic (mGluR), 142–143, 144*t*
  - pathophysiology and pharmacology of, 143–147
    - antagonists, 157
    - in brain trauma, 144–145, 145*f*, 146*f*
    - in epilepsy, 145, 147
    - in hyperalgesia, 145, 146*f*
    - in neurodegenerative diseases, 147
    - in stroke, 144–145, 145*f*, 146*f*
  - physiology of, 140–143
- Glutamate-gated chloride channels, potentiation of, in ivermectin action, 598
- Glutamic acid decarboxylase, in GABA degradation, 147
- Glutaminase, in glutamate synthesis, 141, 141*f*
- Glutathione, glucose-6-phosphate dehydrogenase and, 593
- Glutathione conjugation
  - of alkylating agents, 526
  - in drug metabolism, 48*t*, 51*f*
- Glyceryl trinitrate. *See* Nitroglycerin (NTG)
- Glycine
  - as neurotransmitter, 80
  - in pain transmission regulation, 232
  - structure of, 83*f*
- Glycine conjugation, in drug metabolism, 48*t*, 49, 51*f*
- Glycohemoglobin (HbA1c), in diabetes mellitus, 465
- Glycol ethers
  - absorption of, 721
  - toxicity of, 734*t*
- Glycopeptides (vancomycin and teicoplanin), 568*f*, 572, 577
- Glycoprotein(s)
  - in drug resistance, 529
  - in platelet adhesion, 336, 337*f*
- Glycoprotein IIB-IIIa
  - antagonists of, 348, 390
  - in platelet activation, 339*f*
  - in platelet aggregation, 338, 339*f*
- Glycosides, cardiac, 291, 293–295, 293*f*, 294*t*, 298, 398
- GM-CSF (granulocyte-monocyte colony stimulating factor)
  - recombinant, for neutropenia, 658–659, 661, 774
  - in white blood cell production, 658–660
- GnRH (gonadotropin-releasing hormone), 84, 405, 405*t*, 409*f*, 444, 444*f*
  - agonists of, 449, 455
  - analogues of, 409–410
- Goiter, 418
- Goitrogens, for hyperthyroidism, 420*f*, 421, 423
- Goldman-Hodgkin–Katz equation, 62–63
- Gonadal hormones. *See* Reproductive hormones; *specific hormones*
- Gonadorelin, 414
- Gonadotrophs, 405*t*, 408–410, 409*f*
- Gonadotropin(s). *See also* Follicle-stimulating hormone (FSH); Luteinizing hormone (LH)
  - expression of, modulators of, 409–410, 414
- Gonadotropin-releasing hormone (GnRH), 84, 405, 405*t*, 409*f*, 444, 444*f*
  - agonists of, 449, 455
  - analogues of, 409–410, 784
- Good Manufacturing Practice regulations, 798, 800
- Goodpasture's syndrome, 670*t*
- Goserelin, 410, 414
- Gout, 709–715
  - case study of, 709–710
  - drugs for, 712–715
    - carbonic anhydrase inhibitors, 312
    - colchicine, 531
    - leukocyte suppressors, 712–713, 713*f*, 715
    - urate concentration reducers, 713–715, 713*f*
  - pathophysiology of, 711–712, 711*t*, 712*f*
  - purine metabolism and, 710–711, 710*f*
- gp41 protein, HIV, 547–548, 548*f*, 549*f*
- gp120 protein, HIV, 548*f*
- G6PD (glucose-6-phosphate dehydrogenase) deficiency, primaquine toxicity in, 593
- Graded dose-response relationships, 19, 19*f*
- Graft-versus-host disease, 669
- Gram stain, 566, 566*f*
- Gram-negative bacteria, cell wall of, 566, 566*f*
  - beta-lactam effects on. *See* Beta-lactam antibiotics
  - vs. mycobacterial cell wall, 569
- Gram-positive bacteria, cell wall of, 566, 566*f*
  - beta-lactam effects on. *See* Beta-lactam antibiotics
  - glycopeptide effects on, 572
- Grand mal (tonic–clonic) seizures
  - definition of, 196*t*
  - drugs for, 203–204
  - pathophysiology of, 196–197, 198*f*
- Granules, platelet, 336, 337*f*, 338, 338*f*
- Granulocyte(s), 618, 656, 656*t*
  - production of, 659–661
  - types of, 659–661
- Granulocyte colony-stimulating factor (G-CSF)
  - in hematopoiesis, 658–660
  - recombinant, for neutropenia, 662–663, 665, 774
- Granulocyte-monocyte colony stimulating factor (GM-CSF)
  - in hematopoiesis, 658–660
  - recombinant, for neutropenia, 663, 665, 774
- Granulocyte-stimulating factors, in white blood cell production, 659–660

Granulosa cells, hormone action on, 445, 445*f*
Grapefruit juice effect, in drug metabolism, 53
Graves' disease, 418, 419*f*
Gray baby syndrome, 53, 541
Gray matter, spinal cord, 77, 77*f*
Griseofulvin, 531, 534
Growth factors
  as drugs, 752, 752*t*, 761, 773–777
  hematopoietic, 656–658, 657*f*
  in inflammation resolution, 625
  in white blood cell production, 659–661
Growth fraction, in carcinogenesis, 499
Growth hormone (GH), 404, 752*t*
  deficiency of, 403, 406–407, 407*f*
  in hypothalamic-pituitary-growth hormone axis, 406–407, 407*f*
  inhibitors of, 407, 413
  recombinant, 406, 773
  replacement of, 406–407, 407*f*, 413
Growth hormone-releasing hormone (GHRH), 84, 405–408, 405*t*, 407*f*
  recombinant, 761, 779
Guanabenz, 115, 119, 381
Guanadrel, 113, 118
Guanethidine, 118
  action of, 113, 113*f*
  for hypertension, 381
Guanfacine, 115, 119
Guanine
  alkylation of, 524–525, 525*f*
  in nucleotides, 506, 507, 509*f*, 520, 520*f*
  in purine metabolism, 710, 710*f*
  structure of, 507*t*, 514*f*, 520, 520*f*, 521*f*
Guanosine diphosphate
  in drug-receptor interactions, 9, 10*f*
  structure of, 507*t*
Guanosine monophosphate, in drug-receptor interactions, 9, 10*f*
Guanylate, structure of, 507*t*
Guanylyl cyclase
  in drug-receptor interactions, 9, 11*f*
  receptor, 12
  in vascular tone regulation, 320, 320*f*
  in volume regulation, 303, 303*f*
Gutless adenoviral vectors, 769

**H**

H1 receptor (histamine)
  actions of, 649, 649*t*
  antagonists of, 650–652, 652*f*, 653*f*, 654
H2 receptor (histamine)
  actions of, 649, 649*t*
  antagonists of, 652, 653*f*, 654
H3 receptor (histamine)
  actions of, 649, 649*t*
  antagonists of, 652
HAART (highly active antiretroviral therapy), 556*b*, 608
Half-life ($t_{1/2}$), 39, 39*t*, 40*b*, 43*t*
Hallucinations
  in levodopa therapy, 171
  in schizophrenia, 170
Halofantrine, 591*f*
Haloperidol, 177
  pharmacokinetics of, 174
  for schizophrenia, 171
  structure of, 173*f*
Halothane, 227
  analgesic index of, 207
  autoimmune disease from, 724
  blood/gas partition coefficient of, 214*f*
  delivery of, ventilation effects on, 216*f*
  oil/gas partition coefficient of, 210*f*, 220
  pharmacology of, 220
  properties of, 208*t*
  recovery from, 219, 219*f*
  structure of, 222*f*
  tissue equilibration of, 214*f*
  tissue/blood partition coefficients of, 213*t*
  toxicity of, 734*t*
  volume capacity for, 211*f*
Haploid stages, 595
Haptens, as allergens, 723, 723*f*, 724
Hashimoto's thyroiditis, 418–419, 419*f*
Hatch–Waxman Act, 793*t*, 798
Hay dust, toxicity of, 734*t*
HbA1c (glycohemoglobin), in diabetes mellitus, 465
HBsAg vaccine, 779
HDL. *See* Lipoprotein(s), high-density (HDL)
Headache
  migraine, 237
  in nitrate tolerance, 325
Health Canada, 798
Heart. *See also* Myocardium; *subjects starting with* Myocardial
  abnormal rhythms of. *See* Antiarrhythmic agents; Cardiac arrhythmia(s)
  electrical physiology of, 268–271. *See also* Antiarrhythmic agents; Cardiac arrhythmia(s)
    abnormal, 271–274, 273*f*, 274*f*
    action potentials in, 268–270, 269*f*, 270*t*, 271*b*, 271*f*, 272*f*
    firing rate determination in, 270–271
    nonpacemaker cells in, 268, 272
    pacemaker cells in, 268
  endothelial injury in, thrombosis in, 343
  failure of. *See* Congestive heart failure (CHF); Heart failure
  fibrosis of, aldosterone-mediated, 436
  function of, hypertension and, 378
  hypertrophy of, in congestive heart failure, 394
  ischemic disease of. *See* Ischemic heart disease
  neuregulins of, 297
  valvular disease of, heart failure in, 391
Heart failure
  α-adrenergic antagonists for, 116–117, 117*t*, 119
  congestive. *See* Congestive heart failure (CHF)
  diastolic, 290
  drug half-life in, 39
  drug metabolism in, 54
  loop diuretics for, 312
Heart rate
  β-adrenergic receptor antagonist effects on, 387–388
  as blood pressure determinant, 377, 377*f*
  as contractility determinant, 286, 393
  in myocardial oxygen demand, 318
Heat
  as classic sign of inflammation, 617
  nociceptor firing and, 122, 124*f*
  pain sensitivity to, 230–231, 231*f*
Heavy metals
  chelation of, 740–741, 741*f*
  nephrotoxicity of, 727
*Helicobacter pylori,* in peptic ulcer disease, 685–686, 686*f*, 691
Helminths
  case study of, 597
  drugs for, 598–599, 602
  physiology of, 597–598
Helper T cells, 620*t*, 622, 622*f*
  in asthma, 697–698, 697*f*
Helper-dependent adenoviral vectors, 769
Hemagglutinin, of influenza virus, amantadine effects on, 549
Hematocrit, 658
Hematologic toxicity, of amphotericin B, 583
Hematopoiesis, 655–666
  drugs for, 661–666
    antineoplastic, 663–664, 666
    antineutropenic agents, 655–656, 662–663, 665
    erythropoietic agents, 661–662, 665
    fetal hemoglobin-inducing agents, 662, 665
    platelet-inducing agents, 663, 666
    protein-based, 774
  pathophysiology of, case study of, 655–656
  physiology of, 656–663
    cells, 656, 656*t*, 657*f*
    erythropoiesis, 658–659, 659*f*, 660*t*
    growth factors, 656–658
    platelet production, 661, 661*f*
    white blood cell production, 659–661
Hematopoietic growth factors, 656–658, 657*f*
Heme group, 658
Heme metabolism, in malarial parasites, 589–590, 589*f*
  inhibitors of, 590–592, 591*f*, 592*f*, 600
Hemeprotein monooxygenases. *See also* Cytochrome P450 enzymes
  in drug metabolism, 47
Hemicholinium-3, 90, 91*f*, 98, 104
Hemodialysis, for toxin elimination, 739–740
Hemofiltration, for toxin elimination, 739–740
Hemoglobin
  carbon monoxide binding to, 724, 726*f*
  in erythropoiesis, 658
  malarial parasite ingestion of, 589–590, 589*f*
Hemoglobin A, 658
Hemoglobin F, 658
  inducers of, 662, 665
Hemoglobin S, 658
Hemoglobinopathies, 658
Hemolysis, in glucose-6-phosphate dehydrogenase deficiency, 593
Hemolytic anemia, autoimmune, 670*t*
Hemoperfusion, for toxin elimination, 739–740
Hemophilia, 344*b*
Hemorrhagic disorders, 344, 344*b*
Hemorrhagic shock, anesthesia induction in, 216
Hemostasis
  defects of. *See also* Thrombosis
    hypocoagulability in, 344, 344*b*
  definition of, 335
  modulators of, 345–356
    anticoagulants, 349–353, 349*f*, 351*f*, 352*t*, 355–356
    antiplatelet agents, 326, 345, 346*f*, 347*f*, 348, 355
    protein-based, 775
    thrombolytic agents, 353–354, 356
  physiology of, 336–343
    primary, 336, 337*f*–340*f*, 338
    regulation, 340–343, 342*f*, 343*f*
    secondary, 336, 337*f*, 338–340, 341*f*, 342*f*
    sequence of events in, 336, 337*f*
    vasoconstriction in, 336, 337*f*
Hemozoin, in heme metabolism, 589*f*, 590
Henderson–Hasselbalch equation, 29
Henry's Law, 209*b*, 226
Heparin, 348–350, 355, 752*t*
  action of, 342*f*
  antagonists of, 350
  clinical uses of, 350
  laboratory monitoring of, 350
  low molecular weight, 349–350, 349*f*, 355
  mechanism of action of, 349–350, 349*f*
  structure of, 348–349, 349*f*
  thrombocytopenia induced by, 345, 350
  toxicity of, 350

- Heparin (*contd.*)
  - unfractionated, 349, 349f, 350, 355
  - for unstable angina, 389
- Hepatic lipase, 360, 362, 364f
- Hepatitis, toxic, 726
- Hepatitis B
  - antiviral drugs for, 551f, 554–555, 563
  - vaccine for, 761, 779
- Hepatitis C, antigens of, 779
- Hepato–renal reflex, in ascites formation, 308–309, 309f
- Heroin, addiction to, 256–257
  - case study of, 247–248
  - treatment of, 259, 260f
- Herpes simplex virus
  - drugs for, 552–553, 551f
  - life cycle of, 546
- Herpesviruses
  - antiviral drugs for, 551f, 552–554, 551f, 553f, 562
  - as gene therapy vectors, 766t, 769–770
- HERVs (human endogenous retrovirus sequences), 767
- Heterologous desensitization, 14, 15t
- HETEs (hydroxyeicosatetraenoic acids), 632, 633t, 634f, 635f
- Hexamethonium, 94, 97f
  - action of, 109
  - for hypertension, 381
- Hexarelin, 407, 413
- Hexose transporter, 85–86
- HGPRT (hypoxanthine-guanine phosphoribosyl transferase), 513
  - in purine synthesis, 710–711, 710f
- High altitude illness, acetazolamide for, 312
- High-density lipoprotein. *See* Lipoprotein(s), high-density (HDL)
- Highly active antiretroviral therapy (HAART), 556b, 608
- High-throughput screening, in drug design, 751
- Hippocampal formation, 76
- Hippocampus, 76
  - dopamine receptors in, 164, 165f
- Hirudin, 335, 356, 776
- Hispanic population, hypertension management in, 383
- Histamine, 80, 623, 647–654
  - actions of, 80, 82, 648–649, 648t
  - in anaphylaxis, 651
  - in asthma, 697f, 698
  - degradation of, 648–649, 648f
  - in gastric acid secretion, 684–685, 685f
  - pathophysiology of, 649–651, 650f
  - receptors for, 649, 649t
    - antagonists of, 651–654, 651t, 652f, 653f, 688–690, 688f, 690b, 693
    - in gastric acid secretion, 684, 685f
  - release of, 647–648, 648f
  - storage of, 647–648, 648f
  - structure of, 83f
  - synthesis of, 81, 82, 648–649, 648f
- Histidine decarboxylase, in histamine synthesis, 647, 648f
- Histofluorescence, in dopaminergic neuron localization, 164
- Histrelin, 410, 414
- Hit, in drug discovery, 748, 751
- Hit-to-lead development, 751
- HIV. *See* Human immunodeficiency virus and infection
- Hives (urticaria), 650
- HMG CoA reductase
  - in cholesterol synthesis, 360, 363f
  - inhibitors of (statins), 368–369, 369f, 373, 389
  - polymorphism in, 805–806
- Hodgkin's disease, combination therapy for, 611–612, 612t
- Holoenzymes, in transcription, 536, 536f
- Homeostasis, volume. *See* Volume regulation
- Homologous desensitization, 14, 15t
- Homovanillic acid (HVA), as dopamine metabolite, 163, 164f
- Horizontal transmission, of drug resistance, 501
- Hormone(s). *See also specific hormones*
  - adrenal, 425–440
  - counter-regulatory, 458–459, 458t, 460f
  - as drugs, 752, 752t
  - hypothalamic, 404–405, 405t
  - pancreatic. *See* Diabetes mellitus; Glucagon; Insulin; Somatostatin
  - pituitary, 404–405, 405t
  - reproductive, 441–456
  - thyroid, 416–418, 416f–419f
- Hormone replacement therapy, 453–454, 483–484, 488
- Hormone-sensitive lipase, niacin effects on, 371, 371f
- Housekeeping activities, of cyclooxygenase, 629
- HPETEs (hydroperoxyeicosatetraenoic acids), 632, 633t, 634f, 635
- 5HT. *See* Serotonin
- Human chorionic gonadotropin
  - recombinant, therapy with, 774
  - secretion of, 446
- Human endogenous retrovirus sequences (HERVs), 767
- Human immunodeficiency virus and infection
  - antiviral drugs for
    - attachment and entry inhibitors, 547–548, 549f, 562
    - case study of, 545–546
    - combination, 556b, 608
    - genome replication inhibitors, 549–550, 551f, 562
    - maturation inhibitors, 547f, 556–557, 558f, 559b, 564
    - nucleoside analogues, 551f, 554–555, 555t, 556b, 563
    - reverse transcriptase inhibitors, 555–556, 557b, 557f, 564
    - targets of, 497–498, 497f
  - fungal infections in, 579–580
  - immunoassays for, 761
  - life cycle of, 546–547, 548f
- Humanization, of monoclonal antibodies, 677, 703
- Humoral immunity, 580, 622
- Humoral rejection, acute, in transplantation, 668–669, 668t
- Huntington's disease, gene therapy for, 763
- HVA (homovanillic acid), as dopamine metabolite, 163, 164f
- Hydralazine, 329, 333
  - autoimmune disease from, 724
  - for congestive heart failure, 398
  - for hypertension, 381
  - toxicity of, 734t
- Hydrochloric acid
  - macromolecular damage from, 722
  - toxicity of, 734t
- Hydrochlorothiazide, 313, 316
  - for congestive heart failure, 395–396
  - for hypertension, 379
- Hydrocodone, 239, 244
- Hydrocortisone. *See* Cortisol (hydrocortisone)
- Hydroflumethiazide, 316
- Hydrofluoric acid
  - macromolecular damage from, 722
  - toxicity of, 734t
- Hydrogen bonding, 5, 5t
- Hydrogen ion ATPase, 163, 163f
  - in neurotransmitter synthesis, 182, 182f
  - vacuolar, in volume regulation, 305, 305f
- Hydrolysis, in drug metabolism, 47t
- Hydroperoxyeicosatetraenoic acids (HPETEs), 632, 633t, 634f
- Hydrophilic drugs
  - absorption of, 31
  - phase I reactions producing, 46–47
  - phase II reactions producing, 49, 51f
- Hydrophilic protein segments, 5
- Hydrophilic substances, transport across blood–brain barrier, 85–86, 85f
- Hydrophobic drugs
  - absorption of, 31
  - local anesthetics as, 124, 127f
  - metabolism of, 35
  - parenteral administration of, 31–32
- Hydrophobic protein segments, 5
- Hydrostatic pressure, capillary, volume regulation and, 300, 301f
- Hydroxyapatite, in bone, 474
- α-Hydroxycholecalciferol, 486, 489
- Hydroxyeicosatetraenoic acids (HETEs), 632, 633t, 634f, 635f
- Hydroxy-itraconazole, 582
- Hydroxylation, in drug metabolism, 46t
- 17-Hydroxypregnenolone, 443f
- 11β-Hydroxysterol dehydrogenase, in glucocorticoid metabolism, 427, 428f
- 5-Hydroxytryptamine. *See* Serotonin
- 5-Hydroxytryptophan, in serotonin synthesis, 180, 181f
- Hydroxyurea, 506f, 509f, 510f, 514–515, 517, 610t
  - for sickle cell disease, 662, 665
- Hyperacute rejection, in transplantation, 668, 668t
- Hyperaldosteronism, primary, 436
- Hyperalgesia, 235
  - NMDA receptors in, 145, 146f
- Hyperbaric oxygen chamber, for carbon monoxide poisoning, 742
- Hypercalcemia
  - familial hypocalciuric, 481
  - loop diuretics for, 312
  - of malignancy, 485, 487
  - treatment of, 486–487
- Hypercholesterolemia, 364–365, 365t
  - case study of, 357–358
  - drugs for
    - bile acid absorption inhibitors, 367–368, 373
    - cholesterol absorption inhibitors, 368, 373
    - cholesterol synthesis inhibitors, 368–369, 369f, 373
    - fibrates, 369–370, 370f, 373
    - National Cholesterol Education Program guidelines for, 367, 367t
    - niacin, 370–371, 371f, 373
  - familial, 364–365, 365t
  - in mixed hyperlipidemia, 366
  - secondary, 366–367, 366t
- Hypercholesterolemia, familial, gene therapy for, 763, 763t
- Hypercoagulability, 343–345, 343f, 344b, 344t
- Hyperglycemia, in diabetes mellitus, 463, 464
- Hyperinsulinemia
  - pathophysiology of, 465
  - treatment of, 468–469, 471
- Hyperkalemia
  - from angiotensin-converting enzyme inhibitors, 310
  - arrhythmias in, 282
  - loop diuretics for, 312
  - metabolic acidosis in, from diuretics, 313

Hyperlipidemia, secondary, 366–367, 366*t*
Hyperparathyroidism
  in kidney failure, 483, 483*f*
  loop diuretics for, 312
  primary, 480–481, 480*t*
  secondary, 432, 481
Hyperphosphatemia
  aluminum hydroxide for, 486
  in kidney failure, 483, 483*f*
Hyperpolarization
  in action potential, 64*f*
  in cardiac cells, 279, 280*f*
  of cell membrane, 60
  in GABA$_B$ receptor activation, 147–148, 148*f*
Hyperreactivity, airway, in asthma, 696–699, 698*f*
Hypersensitivity
  IgE-mediated, histamine in, 649–650, 650*f*
  to local anesthetics, 133
  to toxins, 722–724, 723*f*
  types of, 722–724, 723*f*
Hypertension, 376–384
  case study of, 317–318, 376
  classification of, 376, 377, 377*t*
  clinical management of, 378–383. *See also* Antihypertensive agents
    demographic factors in, 383
    in hypertensive crisis, 383
    monotherapy in, 376, 382
    neurohormonal modulation in, 382
    overview of, 378, 379*f*, 379*t*
    stepped care in, 382–383
    sympathetic tone down-regulation in, 380–381
    vascular tone modulation in, 381
    volume reduction in, 378–380
  heart failure in, 391, 392*t*
  isolated systolic, 376
  malignant, 383
  pathophysiology of, 376–378, 377*f*, 377*t*
  primary (essential), 377
  pump-based, 378
  secondary, 377
  vascular resistance-based, 378
  volume-based, 378
Hypertensive crisis, 188, 383
Hypertensive emergency, 383
Hypertensive urgency, 383
Hyperthermia, malignant, 220
Hyperthyroidism
  case study of, 415–416
  drug metabolism in, 54
  drugs for, 420–421, 420*f*, 423
  goiter in, 418
  increased metabolism in, 417–418
  pathophysiology of, 418–419, 419*f*
Hypertriglyceridemia, 365–366, 365*t*
  secondary, 366–367, 366*t*
Hypertrophy, cardiac, in congestive heart failure, 394
Hyperuricemia
  in gout, 711–712, 711*t*, 712*f*
  from niacin, 371
Hyperventilation, anesthetic delivery and, 216, 216*f*
Hypnotics, benzodiazepines as, 154, 154*t*
Hypnozoites, in malaria parasite life cycle, 588*f*, 589
Hypoalbuminemia, edema in, 309, 312
Hypoaldosteronism, 436
Hypocalcemia, treatment of, 487
Hypoglycemia, in hyperinsulinemia, 465
Hypogonadism, 448
Hypokalemia
  arrhythmias in, 282
  digoxin dose in, 293–294
  from loop diuretics, 312
Hypokinesia, in Parkinson's disease, 166–167
Hypomagnesemia, from loop diuretics, 312
Hypomanic episode, in bipolar disorder, 184
Hypoparathyroidism, 480*t*, 481, 486
Hypophosphatemic vitamin D-resistant rickets, 480, 480*t*
Hypophysis. *See* Pituitary gland
Hypotension
  from angiotensin-converting enzyme inhibitors, 310
  from nesiritide, 311
  orthostatic, from tricyclic antidepressants, 187
  reflex tachycardia in, 323
Hypothalamic–pituitary portal vascular system, 404, 404*f*
Hypothalamus
  anatomy of, 76
  axes of
    pituitary-adrenal, 406*f*, 408, 409*f*
    pituitary-growth hormone, 406–407, 407*f*
    pituitary-prolactin, 407–408
    pituitary-reproductive, 408–410, 409*f*, 444–445, 444*f*, 445*f*, 447–448
    pituitary-thyroid, 406–410, 407*f*, 409*f*, 418, 419*f*
  dopamine receptors in, 164, 165*f*
  dopaminergic neurons in, 166, 166*f*
  dysfunction of, 403, 405–406
  function of, 76
  physiology of, 404–406, 404*f*, 406*f*
Hypothyroidism
  from amiodarone, 421–422
  case study of, 416
  from corticosteroids, 422
  cretinism in, 417
  diagnosis of, 408
  drugs for, 419–420, 420*f*, 423
  hyperlipidemia in, 367
  from lithium, 421
  pathophysiology of, 418–419, 419*f*
Hypoventilation, anesthetic delivery and, 216, 216*f*
Hypovolemia, heart failure in, 392*t*
Hypoxanthine-guanine phosphoribosyl transferase, 513
  in purine synthesis, 710–711, 710*f*
Hypoxia
  definition of, 318
  diffusion, in general anesthesia, 219
  erythropoietin production in, 658–659, 659*f*, 660*t*

**I**

$^{131}I^-$ (radioactive iodide), 420–421
I bands, of myocytes, 287*f*
Ibritumomab tiuxetan, 613
Ibuprofen, 240, 244, 639*f*, 640, 644
Ibutilide, 280
ICAMs (intercellular adhesion molecules), in inflammation, 624, 625*f*, 633
Idiosyncratic effects, genetic factors in, 808–809, 808*t*
IDL (intermediate-density lipoprotein), 358*t*, 359–360, 360*t*, 364*f*
Idoxuridine, 562
IECs (Independent Ethics Committees), 793
Ifosfamide, 526
IgE. *See* Immunoglobulin E
Imatinib
  binding of, 5–6, 6*f*, 10
  for chronic myeloid leukemia, 4, 10
  selectivity of, 15
Imidazoleacetic acid, as histamine metabolite, 647, 648*f*
Imipenem, 575, 578
Imipramine, 118, 191
  action of, 114
  for mood disorders, 184–186
  for pain, 241, 245
Immediate hypersensitivity, 722–723, 723*f*
Immune complex-mediated hypersensitivity, 723*f*, 724
Immune system. *See also* Inflammation
  definition of, 617
  diversity in, 621
  fundamental role of, 618
  overview of, 618–622, 619*f*, 620*t*, 621*f*, 622*f*
  response of, to toxins, 722–724, 723*f*
  tolerance in, 621
Immunity
  adaptive, 618, 620–622, 620*t*, 621*f*, 622*f*, 659
  cell-mediated, 580, 622, 622*f*
  cells in, 618–620, 619*f*, 659
  humoral, 580, 622
  innate, 618–620, 620*t*
    cells in, 659
    in fungal defense, 580
Immunoadhesins, 760, 777–779
Immunodeficiency, synergistic antimicrobial combinations in, 606–607
Immunoglobulin E
  in hypersensitivity, 649–650, 650*f*
  monoclonal antibody, for asthma, 703
Immunoregulation, protein-based drugs for, 774–775
Immunosuppressive drugs, 667–681
  antibodies, 676–677, 681
  for autoimmune diseases, 679*t*
  cell adhesion blockers, 679, 681
  costimulation inhibitors, 677–679, 678*f*, 681
  cytokine inhibitors, 674–676, 675*f*, 676*f*, 681
  cytotoxic agents, 671–673, 671*f*–673*f*, 672*t*, 680
  gene expression inhibitors, 670–671, 680
  mechanisms of, 669–670, 671*f*
  pathophysiologic considerations in, 668–669, 668*t*, 670*t*
  specific lymphocyte signaling inhibitors, 674, 674*f*, 675*f*, 680
  for transplantation, 679*t*
  utility of, 679*t*
Impulse conduction. *See* Conduction system
Impurity profile, in drug development, 754
Inactivated ion channels, 8, 65–66
Inactivated state, of sodium ion channels, 194, 195*f*
Inactivation
  in drug resistance, 502
  of poisons, 740–741, 741*f*
  of receptors, 14, 15*t*
Inactive drugs, definition of, 45
Inactive prodrugs, definition of, 45
IND (Investigational New Drug) application, 794–795, 794*f*
Indapamide, 316
Independent Ethics Committees (IECs), 793
Indinavir, 556, 564
Indirect pathway, nigrostriatal, 166, 167*f*
Indirect sympathomimetics, 113–114, 113*f*
Indolamines, 81. *See also* Serotonin
Indole acetic acid derivatives, 240
Indomethacin, 240, 244, 639*f*, 640, 644
Induced fit principle, 5–6

Induction
  of general anesthesia
    cardiac output and, 216–217, 216f
    control of, 217–218, 218f
  of cytochrome P450, 49, 51, 51t, 52b
Induction therapy, for immunosuppression, 677
Infection(s). *See also* Antifungal drugs; Antimicrobial drugs; Antiviral drugs
  gene therapy for, 764
  severe, combination therapy for, 607–608
  susceptibility to, in glucocorticoid therapy, 432
Inferior cervical ganglion, 72
Inferior mesenteric ganglion, 72, 74f
Infertility, female
  in endometriosis, 448
  in polycystic ovarian syndrome, 447–448
  in prolactinoma, 448
Infiltration anesthesia, 132
Inflammation
  airway, in asthma, 637, 697–698, 698f
  in autoimmune diseases. *See* Autoimmune diseases
  in cancer, 638
  case study of, 618
  chemical mediators of, 622–624, 623t. *See also specific substances*
  chronic, 625
  classic signs of, 617
  definition of, 617
  diseases of, 617
  eicosanoids in. *See* Arachidonic acid; Eicosanoids; *specific substances*
  in glomerulonephritis, 637
  glucocorticoid effects on, 428, 429f, 432
  in gout, 711–712, 711t, 712f
  histamine in. *See* Histamine
  in inflammatory bowel disease, 637
  normal response in, 617
  response in, 624–625, 625f
  in rheumatoid arthritis, 637
  from toxins, 722–724, 723f
Inflammatory bowel disease
  inflammation in, 637
  tumor necrosis factor α inhibitors for, 675
Infliximab, 641, 645, 675–676, 676f, 681, 760, 777, 797
Influenza
  ion channel blockers for, 547f, 548–549, 550f
  neuraminidase inhibitors for, 497, 547f, 557, 559–560, 559f, 564
  viral life cycle in, 546
Informed consent, for clinical drug investigations, 793
Inhalants, abuse of, 248t
Inhalation
  of asthma medications, 701, 703–704, 707–708, 782–783
  delivery systems for, 782–783
  formulations for, 756t
  of glucocorticoids, 433–434, 434f
  of toxins, 721, 726
Inhaled anesthetics
  abbreviations related to, 225
  adjuvant drugs with, 221
  analgesic index of, 207, 208f
  balanced, 221
  case study of, 205–206
  cerebral cortical effects of, 75
  discovery of, 205
  drugs for, 227–228. *See also specific drugs*
  equations involved in, 226
  ion channel effects of, 222–223, 223f
  mechanism of action of, 221–223, 222f
  Meyer–Overton rule for, 207, 209b, 210f, 222
  minimum alveolar concentration of, 206–207, 207b, 208t
  partition coefficients of, 207, 209b
  perfusion-limited, 214, 214f
  pharmacodynamics of, 206–207, 206f, 207b, 208t
  pharmacokinetics of, 207–219
    physiologic concepts in, 209–210
    recovery, 218–219, 219f
    uptake model of, 210–218, 211f, 212f, 213t, 214f–217f, 218t
  pharmacology of, 220–221, 220f
  symbols related to, 225
  therapeutic index of, 207, 208f
Inhalers, for asthma, 701, 703–704, 782–783
Inherited diseases, gene therapy for, 762–763, 763t
Inhibin, 409, 444f, 445
Inhibition, of cytochrome P450, 49, 51, 51t, 52b
Inhibitory G protein, in cardiac contractility, 291
Inhibitory neurotransmitters, action of. *See* Gamma-aminobutyric acid (GABA)
Inhibitory postsynaptic potentials (IPSPs), 67, 94–95, 147–148
Inhibitory strategies, in gene therapy, 770–771, 770f
Initial concentration, 43t
Initiation stage
  of carcinogenesis, 727
  of transcription, 536, 536f
  of translation, 537, 537f
Innate immunity, 618–620, 620t
  cells of, 659
  in fungal defense, 580
Innate tolerance, 250
Inner medullary collecting duct, in volume regulation, 306–307, 307f
Inosine monophosphate (inosinate), in purine synthesis, 507, 508f, 509f
Inosine monophosphate dehydrogenase, inhibitors of, for immunosuppression, 672–673, 672f, 672t
Inositol-1,4,5-trisphosphate
  in drug-receptor interactions, 9, 11f
  lithium effects on, 189
Inotropic agents, 291–298
  β-adrenergic receptor agonists, 295–296, 295t, 298
  cardiac glycosides, 291, 293–295, 293f, 294t, 298
  for congestive heart failure, 398
  phosphodiesterase inhibitors, 296–298
  positive, definition of, 285
  types of, 291
Inotropy, positive, definition of, 289
Insecticides, toxicity of, 724, 725f
Insomnia, benzodiazepines for, 154, 154t
Inspired partial pressure ($P_I$), of anesthetics, 207, 210–212, 214–218, 214f, 216f, 218f
Institutional Review Boards (IRBs), 793
Insulin, 84, 752t
  abnormalities of. *See* Diabetes mellitus
  biochemistry of, 459, 461f
  cells secreting, 458
  in combination therapy, 468, 469t
  deficiency of
    hyperlipidemia in, 367
    in type I diabetes mellitus, 463–464, 463t
  delivery systems for, 781–782, 785–786
  development of, 749, 752, 758
  discovery of, 457
  in energy homeostasis, 458–459, 458t, 460f
  excess of (hyperinsulinemia)
    pathophysiology of, 465
    treatment of, 468–469, 471
  glucocorticoid effects on, 428
  intolerance of, from niacin, 371
  manufacture of, 758
  metabolism of, 462
  pump for, 785–786
  receptors for, 10, 460–462, 462f
  recombinant, 773
  replacement of, for diabetes mellitus, 466–467, 466t, 470
  resistance to, in type II diabetes mellitus, 463t, 464
  secretagogues for, 467, 470
  secretion of, 458–460, 461f
  sensitizers of, 467–468, 471
  structure of, 459, 461f
  target tissue action of, 459–462, 460f, 462f
Insulin receptor substrate-proteins (IRS-proteins), 461–462, 462f
Insulin theory, of polycystic ovarian syndrome, 448
Insulin-like growth factor 1, 406, 407f, 658
Integrase, of retroviruses, 765, 767f
Integration, in viral life cycle, 546–547, 548f
Integrative pharmacology
  asthma, 695–707
  cardiovascular
    congestive heart failure, 391–399
    hypertension, 376–383
    ischemic heart disease, 384–391
  gout, 709–715
  peptic ulcer disease, 683–694
Intelligent delivery, of drugs, 785–786
Interbridge, in murein synthesis, 567, 568f
Intercalated cells, in volume regulation, 306–307, 307f
Intercellular adhesion molecules (ICAMs), in inflammation, 624, 625f, 633
Interferon(s)
  antineoplastic effects of, 663, 666
  for chronic myeloid leukemia, 3–4
  in hematopoiesis, 660–661
Interferon-α
  for cancer, 610t
  type I, recombinant, 774
Interferon-α-2a, recombinant, 774
Interferon-α-2b, recombinant, 774, 784
Interferon-β-1a, recombinant, 774
Interferon-γ, recombinant, 775
Interictal spikes, in EEG, 195
Interkantonale Kontrollstelle Für Heilmittel, 798
Interleukin(s), 624
  in asthma, 697–698, 697f
  in hematopoiesis, 658, 660–661
Interleukin-1
  inhibitors of, 676, 681
  release of, in amphotericin B therapy, 583
Interleukin-2
  for cancer, 610t
  in hematopoiesis, 660
  recombinant, 775
Interleukin-3, in hematopoiesis, 658
Interleukin-5, in hematopoiesis, 659–660
Interleukin-7, in hematopoiesis, 660
Interleukin-11, recombinant, 661f, 663, 666, 774
Intermediate-density lipoprotein (IDL), 358t, 359–360, 360t, 364f
International Conference on Harmonization, 793
International normalized ratio, 353
Interomediolateral columns, of sympathetic nervous system, 72
Intestinal phase, of gastric acid secretion, 685
Intestine
  parathyroid hormone effects on, 476, 477t, 478f
  vitamin D absorption in, 478–479

Intra-articular administration, of glucocorticoids, 434
Intracellular bacteria, 573
Intracellular concentration, of drugs, reduction of, in resistance, 502
Intracellular enzymes, loss of function in, gene therapy for, 762–763, 763t
Intracellular receptors, 7f, 12–13, 13f
Intracranial pressure, reduction of, mannitol for, 312
Intramuscular drug administration, 31, 31t, 756t
Intrathecal (spinal) drug administration, 31, 31t
  anesthetics, 132
  for fungal infections, 583
Intravascular volume regulation. *See* Volume regulation
Intravenous drug administration, 31–32, 31t
  anesthetics
    agents for, 227–228
    local, 132
    pharmacology of, 220–221, 220f
  formulations for, 756t
  nitroglycerin, 324
Intrinsic pathway, for coagulation, 339, 341f
Intrinsic (chromosomal) resistance, to antibiotics, 573
Inverse agonists, 20, 24
Inverse tolerance, definition of, 249, 249f
Inverted terminal repeat, in adenoviral genome, 769
Investigational New Drug application, 794–795, 794f
Investigator INDs, 794
Iodide
  inorganic, high doses of, for hyperthyroidism, 420, 420f, 423
  radioactive, for hyperthyroidism, 420–421
  in thyroid hormone synthesis, 416, 416f
    inhibitors of, 420–421, 420f, 423
Iodoquinol, 596
Ion channels. *See also specific ion channels*
  acid sensitive, 231
  in action potential, 63–66, 64f, 65f
  anesthetic effects on
    general, 222–223, 223f
    local, 8
  blockers of, for viral infections, 546, 547f, 548f
  as drug receptors, 7–8, 7f, 8t, 9f
  function of, 60–61, 60f
  ligand-gated, anesthetic effects on, 222–223, 223f
  neurotransmitter effects on, 140, 140f
  pharmacology of, 66
  selectivity of, 61–62, 61f, 62t, 63f
  state-dependent block of, 275
  thermosensitive, 230–231
  voltage-gated, in pain transmission, 128, 129f, 129t, 130f, 232, 233f
Ion trapping, for toxin elimination, 739
Ion-exchange resin, for toxin elimination, 740
Ionic interactions, 5, 5t
Ionotropic glutamate receptors
  inhibitory postsynaptic potential generation by, 147–148, 148f
  physiology of, 141–142, 142f, 143t
Iontophoresis, 783
Ipecac syrup, 730, 738
Ipodate, 421, 423
Ipratropium, 105
  for asthma, 700, 706
  clinical applications of, 101
Iproniazid, 114, 118
  case study of, 107–108
  for mood disorders, 184–185, 188
IPSPs (inhibitory postsynaptic potentials), 94–95, 147–148
Irbesartan, 315
IRBs (Institutional Review Boards), 793
Irinotecan, 529, 533
Iron, toxicity of, 734t
Irreversible antagonist, definition of, 21, 21f
Ischemia
  brain, glutamate receptors in, 144–145, 145f, 146f
  definition of, 318
Ischemic heart disease, 384–391. *See also* Angina; Coronary artery disease (CAD); Myocardial infarction
  acute, heart failure in, 392, 392f
  case study of, 384
  classification of, 384, 384f
  clinical management of, 387–391
    antiarrhythmic agents in, 278
    in coronary artery disease, 387–389, 387t
    goals of, 384
    in myocardial infarction, 390–391
    nitrates in, 322–326, 324f, 325f, 331
    in unstable angina, 389–390
  definition of, 318
  pathophysiology of, 384–387, 384f, 386f
Islets of Langerhans, 458
Isocarboxazid, for mood disorders, 188
Isocyanates, toxicity of, 726
Isoetharine, 699, 706
Isoflurane, 227
  blood/gas partition coefficient of, 214f, 215f, 220
  dose–response curve of, 208f
  MAC of, 207
  oil/gas partition coefficient of, 210f, 220
  pharmacology of, 220
  properties of, 208t
  structure of, 222f
  tissue/blood partition coefficients of, 213t
Isoflurophate, 99, 99f
Isolated systolic hypertension, 376
Isoniazid, 149t, 575, 578, 606
  action of, 571f
  autoimmune disease from, 724
  metabolism of
    acetylation rate and, 806–807, 807f
    genetic variations in, 52
  toxicity of, 734t
Isoprostanes, 628f, 633
Isoproterenol, 115, 119, 699, 706
Isosorbide dinitrate, 324–326, 325f, 331
Isosorbide 2-mononitrate, 325f
Isosorbide 5-mononitrate, 324–325, 325f, 331
Itraconazole, 581, 581f, 582
Ivermectin, 597f, 598, 602

J

Jacksonian march, 196
Jak-Stat signaling, in white blood cell production, 659–660
Japan, drug approval by, 798
Joint(s), glucocorticoid injections into, 434
Juxtaglomerular apparatus, renin secretion from, 301, 302f

K

Kainate receptors, physiology of, 141–142, 142f, 143t
Kanamycin, 539, 543
Kappa opioid receptor, 233
$K_d$ (dissociation constant), 18–19, 18f
Kefauver–Harris Amendments to the Food, Drug and Cosmetic Act (1962), 793t
Kernicterus, 49, 511
Ketamine, 227, 241, 245
  for central sensitization, 236
  pharmacology of, 221
  structure of, 222f
Ketoacidosis, diabetic, 463
Ketoconazole, 427f, 435, 435t, 439, 581, 581f, 582
α-Ketoglutarate
  in GABA degradation, 141f, 147
  in glutamate synthesis, 141, 141f
Ketones, as NSAIDs, 639f, 640, 644
Ketoprofen, 640
Ketorolac, 240–241, 640, 644
Kidney
  dysfunction or failure of
    from aminoglycosides, 540
    from angiotensin-converting enzyme inhibitors, 310
    bone homeostasis in, 482–483, 483f
    chronic, 482–483, 483f, 486
    digoxin dose in, 293
    drug half-life in, 39
    erythropoietin deficiency in, 659f
    glomerulonephritis, 637
    hyperparathyroidism in, 481
    hypertension in, 378
    loop diuretics for, 312–313
    parenchymal disease, 378
    pharmacokinetics and pharmacodynamics in, 40b
    stones, 313
    in toxicity. *See* Nephrotoxicity
    from toxins, 727
  erythropoietin production in, 658–659, 659f, 660t
  excretion from, 36–38, 37f
  parathyroid hormone effects on, 476, 477t, 478f
  in volume regulation, 301–307, 302f, 304f, 307f
Kinetics
  of clearance, 38–39, 38f
  of distribution, 34–35, 35f–37f
Kininase II. *See* Angiotensin-converting enzyme (ACE)
Kinins, in pain physiology, 232
Kir subunits, of potassium ion/ATP channel, 459–460, 461f
Krebs cycle
  in GABA synthesis, 141f, 147
  in glutamate synthesis, 141, 141f
  lack of, in protozoal metabolism, 594

L

Labeling, drug, 798, 799f
Labetalol, 116, 117t, 119
  for arrhythmias, 279
  for hypertension, 380
  pharmacodynamics of, 387t
Lactase, therapy with, 758, 773
Lactic acidosis, from biguanides, 468
Lactotrophs, 164, 165f, 405t
Lamellae, bone, in remodeling, 475, 476f
Lamivudine (3TC), 551f, 555, 563
Lamotrigine, 145, 147, 157
  action of, 200f
  for bipolar disorder, 192
  for pain, 241, 245
  for seizures, 201, 203
Langerhans, islets of, 458
Langerhans cells, function of, 659

Lanosterol, in ergosterol synthesis, 580, 581f
Lansoprazole, 689, 690b, 693
Laplace's law, in cardiac remodeling, 394
Larvae, of helminths, 597, 597f
LAs. *See* Local anesthetics; *specific agent*
Latanoprost, 645
Late, slow excitatory postsynaptic potential, 95, 97f
Late-acting growth factors, for hematopoiesis, 658
Latency, of herpesviruses, 552
Latent pacemaker, of heart, 270–271
Laterodorsal tegmental area, in reward pathway, 257, 257f
LCAT (lecithin:cholesterol acyltransferase), 362, 364f
  defects of, 365t, 366
$LD_{50}$ (median lethal dose), 20, 20f
  in dose–response curve, 208f
  of general anesthetics, 207
  in preclinical testing, 794
LDL lipoprotein. *See* Lipoprotein(s), low-density (LDL)
Lead, in drug discovery, 748, 753
Lead (metal) exposure, 721, 729–730
  chelation in, 740–741, 741f
  toxicity of, 734t
Leak channels, 64f, 65
Learned tolerance, 251
Lecithin:cholesterol acyltransferase (LCAT), 362, 364f
  defects of, 365t, 366
Leflunomide, 673, 673f, 680
Legionnaire's disease, 535–536
Leiden mutation, factor V, 344–345
Leishmaniasis, drugs for, 596, 601
Lente insulin, 466t, 467, 470
Lentiviruses, as gene therapy vectors, 767
Lepirudin, 353
Leptin, in energy homeostasis, 458t, 459
Lesch–Nyhan syndrome, HGPRT absence in, 711
Letrozole, 449f, 450, 455
Leukemia
  chronic myeloid, treatment of, 4
  lymphocytic, B-cell, monoclonal antibodies for, 677
Leukocyte(s). *See also specific cells*
  production of, 659–661
  recruitment of, in inflammation, 624, 625f
  suppression of, for gout, 712–713, 713f, 715
Leukotrienes, 628f, 632, 634f
  in asthma, 637, 697f, 698
  degradation of, 633
  in glomerulonephritis, 637
  inhibitors of, 642–643, 642f, 646, 702–703, 702f, 707
  lipoxin balance with, 633
  receptors for, antagonists of, 643, 646
  synthesis of, 345, 346f
    inhibitors of, 642
Leukovorin, 512
Leuprolide, 410, 414, 610t
Levalbuterol, 700, 707
Levamisole, antineoplastic effects of, 664, 666
Levetiracetam, for seizures, 204
Levobupivacaine, 132, 134, 135
Levodopa, 749
  for Parkinson's disease, 76, 147, 168–169, 168f, 176
  as prodrug, 51
Levofloxacin, 529, 534
Levonorgestrel, 452, 456, 784
Levorphanol, 239, 244
Levosimendan, inotropic effects of, 297
Levothyroxine, 419–420, 423
Leydig cells, hormone action on, 445, 445f
LH (luteinizing hormone), 404, 408, 409f, 444–445, 444f, 445f
  in menstrual cycle, 445–446, 446f
LH (luteinizing hormone) hypothesis, of polycystic ovarian syndrome, 448
Library, for drug screening, 755, 755f
Liddle syndrome, hypertension in, 314
Lidocaine, 133, 135
  for arrhythmias, 278, 283
  for pain, 241
  structure of, 126f
  toxicity of, 132
Life cycle
  of malarial plasmodia, 588–589, 588f
  of viruses, 497, 497f, 546–547, 547f, 548f
Lifestyle changes
  for dyslipidemia, 367
  for hypertension, 378
  for peptic ulcer disease, 691
Ligand-binding domain, of ion channels, 8
Ligand-gated ion channels, 7, 8t, 9f, 222–223, 223f
Limbic system, 76
  dopamine receptors in, 164, 165f
Lincosamides, 537f, 539t, 542, 544
Lineage-specific (lineage-dominant) growth factors, for hematopoiesis, 658
Linear synthesis, in combinatorial chemistry, 754–755, 755f
Linezolid, 542, 544
Linoleic acid, in arachidonic acid synthesis, 628
Lipase, therapy with, 773
Lipid(s). *See also* Lipoprotein(s); Triglycerides
  in amphotericin B formulations, 583
  disorders of. *See* Dyslipidemia
  extractable, in mycobacterial cell wall, 566f, 569
Lipid bilayer, 28–29, 29f, 60–61, 60f
  of bacterial cell wall, 566, 566f
Lipid solubility hypothesis, of general anesthetic action, 222
Lipid-lowering agents. *See also* Dyslipidemia, drugs for
  for coronary artery disease, 389
Lipocortins, 638
Lipophilic drugs
  antipsychotics as, 174
  intracellular receptors for, 12–13, 13f
  metabolism of, 35
  tricyclic antidepressants as, 187
Lipophilic substances, transport across blood–brain barrier, 85, 85f
Lipopolysaccharides, of bacterial cell wall, 566, 566f
Lipoprotein(s)
  abnormal levels of. *See* Dyslipidemia
  apolipoproteins associated with, 358t, 359, 359f, 360, 360t
  classes of, 358, 358t
  high-density (HDL), 358t
    apolipoproteins associated with, 360t
    disorders of, 365t, 366
    elevation of, fibrates in, 370, 370f
    metabolism of, 361, 364f, 365t, 366
    receptor for, 362
    role of, 361–363, 363f, 364f
  intermediate-density (IDL), 358t, 359–360, 360t, 364f
  low-density (LDL), 358t
    apolipoproteins associated with, 358t, 359, 360t
    atherosclerosis and, 363, 363f
    in hypercholesterolemia. *See* Hypercholesterolemia
    in mixed hyperlipidemia, 366
    oxidation of, 361, 363, 363f
    receptor for, 360–361, 362f, 363f, 364–365
    reduction of. *See* Dyslipidemia, drugs for
    removal of, 360–361
    synthesis of, 359–360
    VLDL derived from, 360
  structures of, 358–359, 359f
  synthesis of, 359–361, 362f, 363f
  very low-density (VLDL)
    apolipoproteins associated with, 358t, 359, 359f, 360, 360t
    in hypertriglyceridemia, 365–366
    in mixed hyperlipidemia, 366
    reduction of. *See* Dyslipidemia, drugs for
    removal of, 360
    in secondary hyperlipidemia, 367
    structure of, 359f
    synthesis of, 359–360
Lipoprotein lipase
  deficiency of, familial hypertriglyceridemia in, 365t, 366
  fibrate effects on, 370, 370f
  in lipoprotein degradation, 359, 360, 361f
Liposomes, for drug delivery, 782, 786–787
Lipoteichoic acids, of bacterial cell wall, 566f, 569
Lipotropin, 408, 429
Lipoxins, 628f, 632–633, 633t, 635f
  aspirin-triggered, 639, 643
  degradation of, 633
  for inflammation, 643
Lipoxygenase
  *ALOX5* gene, polymorphism in, 805
  in arachidonic acid metabolism, 628f, 631–633, 633t, 634f, 635f
  inhibitors of, 642, 642f, 646
  in leukotriene synthesis, 345, 346f
5-Lipoxygenase activating protein (FLAP), 632, 634f
  inhibitors of, 642, 649
Lisinopril
  for vascular tone regulation, 333
  for volume regulation, 310, 315
Lispro insulin, 466–467, 466t, 470
Lithium
  for bipolar disorder, 180, 185, 189–190, 192
  for SIADH, 410
  thyroid effects of, 421
Liver
  cirrhosis of, edema in, 308–309, 309f
  drug metabolism in, 31, 35, 47, 49f
    disease effects on, 53–54
    phase I reactions, 35, 45–49, 46t–47t, 50f
    phase II reactions, 35, 45–47, 48t, 49, 51f
  failure of, drug half-life in, 39
  as insulin target, 459, 460f, 462
  lipoprotein synthesis in, 359–361, 361f–363f
  toxicity to, 726
    alcohol, 729
    carbon tetrachloride, 722
  vitamin D storage in, 478, 478f
LMW (low molecular weight) heparin, 349–350, 349f, 355
Loading dose, 42
Local anesthetics, 121–135
  administration of, 131–132
  amide-linked, 124, 126f, 131, 131f, 133–135
  amine group, 124–125
  vs. analgesics, 123
  aromatic group, 124, 127f
  case study of, 121–122
  central nerve blockade, 132

  chemistry of, 124–125, 126*f*, 127*f*
  definition of, 121
  ester-linked, 124, 126*f*, 131, 131*f*, 133, 135
  excretion of, 131, 131*f*
  hydrophobicity of, 124, 127*f*
  infiltration, 132
  intravenous regional, 132
  ion channel conductance and, 8
  mechanisms of, 126–130
    anatomic considerations in, 126–128, 127*f*
    receptors, 130
    voltage-gated sodium channels, 128, 129*f*, 129*t*, 130*f*
  metabolism of, 131, 131*f*
  nociception physiology and, 122–123, 123*f*–126*f*, 125*t*
  peripheral nerve blockade, 132
  pharmacokinetics of, 130–131, 131*f*
  protonation of, 124–125, 126*f*
  topical, 131
  toxicity of, 132–133
Local circuit neuronal organization, 78*f*, 79
Localized vasoconstriction, in hemostasis, 336, 337*f*
Locus ceruleus
  anatomy of, 77
  neurons in, 79, 79*t*, 80*f*
  neurotransmitter release from, 180
  stimulant drug interactions with, 257, 258*f*
Log cell kill model, 499–500, 501*f*, 609
Long QT interval syndrome, lidocaine for, 278
Long terminal repeats, of retroviruses, 765–767, 768*f*
Long tract neuronal organization, 78–79, 78*f*
Longer-acting nitrates, 324
Long-term testing, preclinical, 794
Loop diuretics, 312–313, 316, 380
Loop of Henle, in volume regulation, 304*f*, 305–306, 306*f*, 312–313, 316
Lopinavir, 556, 564, 748
Loratadine, 652, 654
Lorazepam, 157, 228
  clinical applications of, 154*t*
  with general anesthesia, 221
Losartan
  for vascular tone regulation, 333
  for volume regulation, 311, 315
Lovastatin, 369, 373
Low molecular weight heparin, 349–350, 349*f*, 355
Low-density lipoproteins. *See* Lipoprotein(s), low-density (LDL); Lipoprotein(s), very low-density (VLDL)
Loxapine, 177
LTs. *See* Leukotrienes
Lung
  drug delivery to, 782–783. *See also* Inhalation
  toxicity to, 726
Lusitropy, positive, 289
Luteal phase, of menstrual cycle, 445, 446*f*
Luteinizing hormone (LH), 404, 408, 409*f*, 444–445, 444*f*, 445*f*
  in menstrual cycle, 445–446, 446*f*
Luteinizing hormone (LH) hypothesis, of polycystic ovarian syndrome, 448
Lyme disease, vaccine for, 779
Lymphocyte signaling inhibitors, for immunosuppression, 674, 674*f*, 675*f*, 680
Lymphocytes, 656, 656*t*. *See also* B cells; T cells
  production of, 659–661
  types of, 659
Lymphocyte-stimulating factors, 660–661
Lymphoid stem cells, 618, 619*f*, 659
Lymphoma
  monoclonal antibodies for, 613
  vaccines for, 761
Lymphopoiesis, 659–661
Lyophilized powders, 756

**M**

$M_2$ (proton channel), of influenza virus, amantadine effects on, 549, 550*f*
Ma huang, 801
MAC. *See* Minimum alveolar concentration (MAC)
mAChRs. *See* Muscarinic acetylcholine receptors (mAChRs)
Macrolides, 537*f*, 539*t*, 541, 544
Macromolecules
  damage of, in toxicity, 722
  in drug design, 752, 755
Macrophage(s), 619–620, 619*f*, 620*t*, 656, 656*t*, 657*f*
  alveolar, in fungal defense, 580
  function of, 659
  production of, 659
Macrophage scavenger receptor, in LDL removal, 360
Magnesium hydroxide, 690, 693
Magnesium sulfate, 487
Maintenance dose, 42–43, 42*f*
Major depressive disorder, 179
  definition of, 183
  diagnostic criteria for, 183*b*
  drugs for, 186–189, 187*f*
  neurotransmission and, 180–183, 181*f*, 182*f*, 183*t*
  pathophysiology of, 183–185
Major histocompatibility complex (MHC), 620–621, 621*f*, 669
Malaria, 587–593
  case studies of, 588
  cerebral, 588, 589
  drugs for, 590–593, 600
    electron transport chain inhibitors, 590*f*, 592–593, 600
    heme metabolism inhibitors, 590–592, 591*f*, 592*f*, 600
    targets of, 498
  epidemiology of, 587–588
  organisms causing, 588–590, 588*f*–590*f*
Malathion, toxicity of, 724, 725*f*, 735*t*
Male contraception, 453
Malignancy. *See also* Cancer
  hypercalcemia of, 485, 487
Malignant hypertension, 383
Malignant hyperthermia, 220
Malignant melanoma
  gene therapy for, 763*t*
  vaccines for, 768
Manic episodes, in bipolar disorder, 179, 184, 184*b*
Mannans, in fungal cell wall, 580
Mannitol, in volume regulation, 312, 316
Manufacture, of drugs, 754–756, 755*f*
  regulations for, 800
MAO. *See* Monoamine oxidase (MAO); Monoamine oxidase inhibitors (MAOIs)
Maprotiline, 241
Marijuana, 248*t*, 259
Market withdrawal, 800, 800*t*
Marketing, of drugs, regulation of, 798–799
Mass spectrometry, in drug development, 754
Mast cells, 620*t*
  in asthma, 697*f*, 698
  degranulation of
    in allergic reaction, 650, 650*f*
    inhibitors of, 701–702, 707
    in nociception, 123*f*
  histamine synthesis in, 647–648
Matrix protein, of influenza virus, amantadine effects on, 549, 550*f*
Maturation, in viral life cycle, 546, 547*f*, 548*f*, 556
Mature onset diabetes in the young (MODY), 464
Maximum tolerated dose, in clinical trials, 795
Mazzotti-type reaction, to ivermectin, 598
MBC (minimum bactericidal concentration), of antimicrobial drugs, 604–605, 604*f*, 604*t*, 605*f*
M-CSF (monocyte colony-stimulating factor), in hematopoiesis, 659–660
MDMA (methylenedioxymethamphetamine), 248*t*, 259
MDR (multiple drug resistance) transporters, 86, 721
Mebendazole, 599, 602
Mecamylamine, 105
  action of, 109
  clinical applications of, 103
Mechanical stimuli, pain response to, 230–231, 231*f*
Mechanistic toxicology, 743
Mechlorethamine, 524, 525*f*, 526, 533
Meclofenamate, 640, 644
Medial forebrain bundles, in reward pathway, 251
Medial septal nuclei, 80*f*
Median effective dose ($ED_{50}$), 20, 20*f*, 25
Median lethal dose ($LD_{50}$), 20, 20*f*
  in dose–response curve, 208*f*
  of general anesthetics, 207
  in preclinical testing, 794
Median toxic dose ($TD_{50}$), 20, 20*f*, 25
Medical review, of new drugs, 795, 796, 797*f*
Medroxyprogesterone acetate, 456
Medulla, anatomy of, 75, 75*f*, 77
Medullary depression stage, of general anesthesia, 206*f*
MEDWatch program, 799
Mefenamate, 639*f*, 640
Mefloquine, 591*f*
Megakaryocytopoiesis, 661, 661*f*
Megestrol acetate, 456
Meglitinides, for diabetes mellitus, 460*f*, 461*f*, 467, 470
Meglumine antimonate, 596, 601
Melanocyte stimulating hormone (MSH), 408, 429
Melanoma, malignant
  gene therapy for, 763*t*
  vaccines for, 768
Melarsoprol, 596, 601
Meloxicam, 641, 641*f*, 644
Melphalan, 524, 526, 527, 533
Memantine, 144, 157
Membrane, cellular. *See* Cell (plasma) membrane
Membrane attack complex, 623
Membrane diffusion, 28–29, 29*f*
Membrane-bound procoagulant factors, 340
Mendelian inherited disorders, gene therapy for, 762–763, 763*t*
Meningitis, ivermectin toxicity in, 598
Menopause, 448
  hormone replacement therapy for, 453–454
  osteoporosis after, 481–482, 482*f*
Menstrual cycle, 445–446, 446*f*, 452
Meperidine, 244
  as anesthetic adjunct, 228
  contaminants of, neurotoxicity of, 727

MEPP (miniature end-plate potential), 94, 96*f*
6-Mercaptopurine, 506*f*, 509*f*, 513–514, 514*f*, 517
  allopurinol interactions with, 713–714, 713*f*
  formation of, 671–672, 671*f*
Mercury
  autoimmune disease from, 724
  ingestion of, 720
  nephrotoxicity of, 727
  toxicity of, 735*t*, 740–741, 741*f*
Meropenem, 575, 578
Merozoites, in malarial parasite life cycle, 588, 588*f*
Mesenteric ganglia, 72, 74*f*
Mesna, 526
Mesocortical system, dopamine dysregulation in, schizophrenia and, 171
Mesolimbic system, dopamine dysregulation in, 171
Mesoridazine, for schizophrenia, 177
Mestranol, 452, 456
Metabolic acidosis, hyperkalemic, from diuretics, 313
Metabolic blood–brain barrier, 86
Metabolism
  drug, 45–54
    case study of, 45–46
    definition of, 27, 28*f*
    factors affecting, 52–54
    increased, tolerance in, 251, 251*f*
    kinetics of, 38–39, 38*f*
    in liver, 31
    of local anesthetics, 131, 131*f*
    pathways of, 47–52
      induction and inhibition, 49, 51, 51*t*, 52*b*
      metabolites, 51–52
      phase I reactions, 35, 45–49, 46*t*–47*t*, 50*f*, 806, 807*t*
      phase II reactions, 35, 45–47, 48*t*, 49, 51*f*, 806, 807*t*
    phase I reactions in, 35, 45–49, 46*t*–47*t*, 50*f*, 806, 807*t*
    phase II reactions in, 35, 45–47, 48*t*, 49, 51*f*, 806, 807*t*
    polymorphisms in, 806–808, 807*f*, 807*t*, 808*f*
    sites of, 46–47, 46*t*–48*t*, 49*f*
  glucocorticoid effects on, 428
Metabolites
  active, definition of, 45
  toxic, 45, 51–52
Metabotropic ATP-sensitive potassium ion channels, 328–329
Metabotropic receptors, 67, 68, 80, 82*t*
  in central sensitization, 236, 236*f*
  glutamate (mGluR), 142–143, 144*t*, 146*f*
Metal-ligand complex, in toxin inactivation, 740
Metaproterenol, 115, 119, 699, 707
Metastasis, 499
Metered-dose inhalers, 701, 703–704, 707–708, 782–783
Metformin, for diabetes mellitus, 468, 471
Methacholine, 95, 104
  action of, 97, 101, 101*t*
  structure of, 100*f*
Methacycline, 540, 543
Methadone, 239, 244, 259, 260*f*, 261
Methamphetamine, 114
Methanol, toxicity of, 727, 735*t*
Methemoglobin
  formation of, 724, 726*f*
  iron reduction in, 742
Methicillin, 573, 577
Methimazole, 421, 423
Methionine, formylated, in translation, 537
Methohexital, 152*t*, 159
Methotrexate, 511–512, 511*f*, 511*t*, 517
  for immunosuppression, 672, 680
  structure of, 510*f*
Methoxamine, 115, 118
Methoxyflurane
  oil/gas partition coefficient of, 210*f*
  recovery from, 219*f*
Methyl mercury, encephalopathy from, 727
Methylation, in drug metabolism, 48*t*, 51*f*
N-Methyl-D-aspartate receptors. *See* NMDA receptor(s)
Methyldopa, 115, 119
  autoimmune disease from, 724
  for hypertension, 381
  toxicity of, 735*t*
Methylene blue, for methemoglobin iron reduction, 742
Methylenedioxymethamphetamine (MDMA), 248*t*, 259
Methylenetetrahydrofolate, in folate synthesis, 508
Methylphenidate, 113, 118
  extended-release, 786
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
  neurotoxicity of, 727
  Parkinson's disease due to, 167
  toxicity of, 735*t*
Methylprednisolone, 439
  potency of, 430, 432*t*
  structure of, 431*f*
Methyltestosterone, 610*t*
α-Methyltyrosine, 112, 118
Methylxanthines, for asthma, 700, 706
Metoclopramide, toxicity of, 724, 735*t*
Metolazone, 316
Metoprolol, 117, 117*t*, 119
  for arrhythmias, 279, 283
  pharmacodynamics of, 387*t*
  for vascular tone regulation, 333
Metronidazole, 595–596, 595*f*, 601, 691
Metyrapone, 427*f*, 435, 435*t*, 439
Mevalonate pathway, inhibitors of, for bone disorders, 485–486
Mexiletine, 245, 278, 283
Meyer–Overton rule, for inhaled anesthetics, 207, 209*b*, 210*f*, 222
Meynert, basal nucleus of, 79–80, 79*t*
Mezlocillin, 573, 575, 577
mGluR (metabotropic glutamate receptors), 142–143, 144*t*, 146*f*
MHC (major histocompatibility complex), 620–621, 621*f*, 669
MIC (minimum inhibitory concentration)
  of antimicrobial drugs, 604–605, 604*f*, 604*t*, 605*f*
  in drug combinations, 605–606, 605*f*
Michaelis–Menten kinetics, 38, 38*f*
Microbiology review, of new drugs, 796, 797*f*
Microchip drug delivery system, 786
Microsomal triglyceride transfer protein, 360
Microsomes, ergosterol synthesis in, 580
Microspheres, 782
Microtubules
  inhibitors of, 530–531, 530*f*, 534
  physiology of, 521, 523, 523*f*, 524*f*
Midazolam, 158, 228
  action of, 153*f*
  clinical applications of, 154*t*
  with general anesthesia, 221
  for seizures, 202
Midbrain, anatomy of, 75, 75*f*
Middle cervical ganglion, 72
Mifepristone (RU-486), 435, 439, 449*f*, 452, 456
Miglitol, for diabetes mellitus, 465–466, 470
Migraine, 237, 241–242
Mild intermittent asthma, management of, 704, 704*t*
Mild persistent asthma, management of, 704, 704*t*
Milrinone
  for congestive heart failure, 397*t*, 398
  inotropic effects of, 296–298
  for vascular tone modulation, 326
Miltefosine, 596, 601
Mineralization, of bone
  disorders of. *See* Bone disorders
  physiology of, 474–479, 474*f*, 475*f*, 476*f*, 477*t*, 478*f*
Mineralocorticoids, 435–437. *See also* Aldosterone; Renin-angiotensin–aldosterone system
  disorders of
    drugs for, 436–437, 439
    excess, 314
    pathophysiology of, 436
  physiology of, 435–436
  receptors for, 428–429
    agonists of, 436, 439
    antagonists of, 436–437, 439
Miniature end-plate potential (MEPP), 94, 96*f*
Minimum alveolar concentration (MAC)
  of anesthetics, 207
  in balanced anesthesia, 221
  vs. oil/gas partition coefficient, 207, 208*t*
Minimum bactericidal concentration (MBC), of antimicrobial drugs, 604–605, 604*f*, 604*t*, 605*f*
Minimum inhibitory concentration (MIC)
  of antimicrobial drugs, 604–605, 604*f*, 604*t*, 605*f*
  in drug combinations, 605–606, 605*f*
Minimum lethal dose, in preclinical testing, 794
Ministry of Health and Welfare (Japan), 798
Minocycline, 540, 543
Minoxidil
  for hypertension, 381
  for vascular tone regulation, 329, 332
Mirtazapine, 189, 191
Misoprostol, 240, 452, 645, 691, 694
Misuse, drug, definition of, 249
MIT (monoiodotyrosine), in thyroid hormone synthesis, 416, 417*f*
Mitochondrial disorders, gene therapy for, 762–763, 763*t*
Mitogens, 728
Mitomycin, 526, 533
Mitosis, 521, 523, 523*f*, 524*f*
Mitotane, 435, 435*t*, 439
Mitotoxicity hypothesis, 499
Mivacurium, 101, 105, 228
Mixed agonist-antagonists. *See* Partial agonists
Mixed alpha-beta adrenergic antagonists, for hypertension, 380
Mixed episodes, in bipolar disorder, 184
Mixed sympathomimetics, 113–114
Mixed venous return, partial pressure of, 215–216, 226
$M_1$–$M_5$ receptors, 91–92, 92*t*
MMA (mycophenolic acid), for immunosuppression, 672–673, 672*f*, 672*t*
MMF (mycophenolate mofetil), for immunosuppression, 672–673, 672*f*, 672*t*
Moclobemide, 114, 118, 188, 191
Moderate persistent asthma, management of, 704, 704*t*
Moderation management, of drug addiction, 260
Modulated receptor hypothesis, for local anesthetic action, 128, 129*t*

MODY (mature onset diabetes in the young), 464
Moexipril, 315
Molds. *See also* Antifungal drugs; Fungi and fungal infections
  definition of, 579
  toxicity of, 735*t*
Molecular target of rapamycin (mTOR), inhibitors of, 674, 675*f*
Molindone, 177
Moloney murine leukemia virus, as gene therapy vector, 768, 768*f*
Monoamine oxidase (MAO)
  in catecholamine metabolism, 110, 110*f*, 182
  in dopamine degradation, 163, 163*f*, 164*f*
  reserpine effects on, 112–113
  in tyramine metabolism, 113
Monoamine oxidase inhibitors (MAOIs), 114, 118, 191
  action of, 82, 187*f*, 188
  case study of, 107–108
  discovery of, 184
  for Parkinson's disease, 169
  pharmacokinetics of, 188–189
  side effects of, 188
  types of, 188
Monoamine theory of depression, 184–185
Monoamine transporters, 182, 182*f*
Monobactams, 568*f*, 572*f*, 573, 575, 578
Monoclonal antibodies, 760, 777–779
  for asthma, 705
  for cancer, 610*t*, 613
  for immunosuppression, 677, 681
Monocyte colony-stimulating factor (M-CSF), in hematopoiesis, 659–660
Monocytes, 656*t*, 657*f*
Monoiodotyrosine (MIT), in thyroid hormone synthesis, 416, 417*f*
Monotherapy, for hypertension, 376, 382
Montelukast, 634*f*, 642*f*, 643, 646, 702*f*, 703, 707
Mood disorders. *See* Affective disorders; *specific disorders*
Moricizine, for arrhythmias, 278
Morning-after contraception, 453
Morphine, 238, 244
  abuse of, 255
  as anesthetic adjunct, 228
  development of, 748
  with general anesthesia, 221
  for pain, 234
  receptors for, 233
  structure of, 750*t*
  tolerance to, 253*f*
  toxicity of, 735*t*
Motor nervous system
  anatomy of, 73*f*, 73, 75
  long tract neuronal organization of, 78–79
Mountain sickness, acetazolamide for, 312
Movement
  control of, 166, 167*f*
  disorders of, in Parkinson's disease. *See* Parkinson's disease
MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
  neurotoxicity of, 727
  Parkinson's disease due to, 167
  toxicity of, 735*t*
MraY, in murein synthesis, 567, 568*f*
MRSA (methicillin-resistant *Staphylococcus aureus*), 574
MSH (melanocyte stimulating hormone), 408, 429
mTOR (molecular target of rapamycin), inhibitors of, 674, 675*f*
Mu opioid receptor, 233, 244
Mucous membrane, drug administration across, 31*t*, 32
Mucus, in stomach, protective effects of, 685
Multilineage (general) growth factors, for hematopoiesis, 658
Multiple drug resistance (MDR) transporters, 86, 721
Multiple ionic currents, 13
Multiple sclerosis, 670*t*
Multiple-dose activated charcoal, 738
MurA (enolpyruvate transferase), in murein synthesis, 567, 568*f*
MurB (NADPH reductase), in murein synthesis, 567, 568*f*
MurC, in murein synthesis, 567, 568*f*
MurD, in murein synthesis, 567, 568*f*
MurE, in murein synthesis, 567, 568*f*
Murein, in bacterial cell wall, 565, 565*f*
  crosslinking of, 567, 568*f*, 569–570, 570*f*
  inhibitors of, 570–572, 571*f*, 577
    crosslinking, 572–575, 572*f*, 574*f*, 577–578
    monomer synthesis, 570–572, 571*f*, 577
    polymerization, 572, 577
  polymerization of, 567, 568*f*
  synthesis of, 567, 568*f*
MurF, in murein synthesis, 567, 568*f*
MurG, in murein synthesis, 567, 568*f*
Muromonab-CD3 (OKT3), 677, 681, 778
Muscarine, 100*f*, 101
Muscarinic acetylcholine receptors (mAChRs), 90–92, 91*f*, 92*t*
  agonists of, 100–101, 100*f*, 101*t*, 104
  antagonists of, 102, 105, 173
  partial agonists and, 23, 23*f*
Muscimol, 149*t*, 150
Muscle(s)
  glucocorticoid effects on, 432
  as insulin target, 459, 460*f*, 462
  local anesthetic damage to, 132
Muscle group, in uptake model, 210, 211*f*, 219
Muscle relaxants, benzodiazepines as, 154, 154*t*
Mutations
  in carcinogenesis, 728
  diseases traced to, gene therapy for, 762–763, 763*t*
  in drug resistance, 501–503, 502*t*
Myasthenia gravis
  acetylcholinesterase inhibitors for, 99–100
  case study of, 89–90
Mycobacteria, cell wall of, 566, 566*f*, 569, 571*f*
*Mycobacterium tuberculosis*. *See also* Tuberculosis
  cell wall of, 570, 570*f*
Mycolic acids, in mycobacterial cell wall, 566*f*, 570
Mycophenolic acid and mycophenolate mofetil, 672–673, 672*f*, 672*t*
Mycoses. *See* Fungi and fungal infections
Myeloid stem cells, 618, 619*f*, 659
Myelopoiesis, 659–661
  agents inducing, 662–663, 665
Myelosuppression
  neutropenia in, drugs for, 662–663, 665
  from vinblastine, 530
Myocardial contractility. *See* Cardiac contractility
Myocardial infarction
  altered automaticity in, 272
  case study of, 384
  clinical management of, 390–391
  gene therapy after, 764
  management after, 390–391
  nontransmural, 386
  pathophysiology of, 386–387
  subendocardial, 386
  transmural, 386
Myocardial ischemia. *See* Ischemic heart disease
Myocardium
  contractile state of. *See* Cardiac contractility
  oxygen demand of
    determinants of, 318, 319*f*, 319*t*
    reduction of, 387–389, 387*t*
  remodeling of, in congestive heart failure, 393–394, 394*f*
Myoclonic seizures, definition of, 196*t*
Myocytes
  anatomy of, 286, 287*f*, 288*f*, 289*t*
  contraction of
    pathophysiology of, 289–291, 292*f*, 293*t*
    physiology of, 286–287, 288*f*
  nonpacemaker, 268, 272
  pacemaker, 268
  ventricular, electrical physiology of, 268–270, 269*f*, 270*t*, 271*f*
Myofibrils, of myocytes, 286, 287*f*, 289*t*
Myopathy, from niacin, 371
Myosin, of myocytes, 286–287, 287*f*, 288*f*, 289*t*
  relaxation-contraction of, 318–320, 319*f*, 320*f*
Myosin light chain kinase, in vascular tone, 319, 320*f*
Myosin light chain phosphatase, in vascular tone regulation, 320, 320*f*
Myotomal distribution, 73

**N**

$N_1$ ($N_M$) receptors, 92*t*, 93
$N_2$ ($N_N$) receptors, 92*t*, 93
Nabumetone, 639*f*, 640, 644
nAChRs. *See* Nicotinic acetylcholine receptors (nAChRs)
Nadolol, 116, 117*t*, 119, 387*t*
NADPH reductase (MurB), in murein synthesis, 567, 568*f*
Nafarelin, 410, 414
Nafcillin, 573, 577
NAG (*N*-acetylglucosamine), in murein synthesis, 567, 568*f*, 569*b*
Naloxone, 239, 244, 263, 741
Naltrexone, 221, 239, 244, 260, 261, 263
NAM (*N*-acetylmuramic acid), in murein synthesis, 567, 568*f*, 569*b*
Naming, drug, 798
NAM-L-alanine amidase, in bacterial cell wall degradation, 569–570
Naproxen, 240, 244, 640
Naratriptan, 241, 245
NAT (N-acetyltransferase), polymorphism in, 806–807, 807*f*
Natalizumab, 681
Nateglinide, 470
National Cholesterol Education Program, dyslipidemia treatment guidelines of, 367, 367*t*
Native pacemaker, of heart, 270
Natriuresis, in volume regulation, 301
Natriuretic peptides
  increased, in congestive heart failure, 308, 308*f*
  in volume regulation, 303, 303*f*
Natural killer cells, 656*t*, 657*f*
Natural products, as drugs, 748–749, 750*t*
NBC1 (sodium/bicarbonate cotransporter), in volume regulation, 305, 305*f*
NCC1 (sodium-chloride cotransporter), in volume regulation, 306, 306*f*
NDA (New Drug Application), 796–797, 797*f*
Necrosis
  in toxicity, 722
  from toxins, 726
Nefazodone, 189, 191

Negative symptoms, in schizophrenia, 170
Nelfinavir, 556, 564
Nematodes
  case study of, 597
  drugs for, 598–599, 602
  physiology of, 597–598
Neomycin, 539, 543
Neonates, drug metabolism in, 53
Neoplasia, drugs for. *See* Antineoplastic drugs
Neostigmine, 104
  action of, 98, 99
  clinical applications of, 100
  for muscle relaxant reversal, 221
  structure of, 99, 99*f*
  toxins resembling, 724
Nephrogenic diabetes insipidus, 410, 411*f*
  lithium-induced, 189
Nephrolithiasis, thiazides for, 313
Nephron, in volume regulation, 304–307, 304*f*–307*f*
Nephrotic syndrome, edema in, 309
Nephrotoxicity, 727
  of aminoglycosides, 540
  of amphotericin B, 583
  of cyclosporine, 674
  of lead, 730
  of platinum compounds, 527
Nernst equation, 61–62, 61*f*, 62*t*, 63*f*
Nernst equilibrium potential, 62, 62*t*, 63*f*, 268, 269*f*
Nerve(s)
  anatomy of, for local anesthetic administration, 126–128, 127*f*
  blockade of, local anesthetics in, 132
  histamine effects on, 648*t*, 649
Nerve fibers, types of, 125*t*
Nerve gases, 721, 724, 725*f*
Nervous system
  anatomy of, 72–80
    autonomic, 72–73, 74*f*
    central, 75–78, 75*f*–77*f*
    motor, 73, 73*f*, 75
    peripheral, 72–75, 73*f*, 74*f*
    sensory, 73, 73*f*, 75
  central. *See* Brain; Central nervous system; Spinal cord
  dysfunction of, case study of, 71–72
Nesiritide, 311, 315, 776
NESP (novel erythropoiesis stimulating protein, darbepoetin), 661–662, 665
NET. *See* Norepinephrine transporter (NET)
Netilmicin, 539, 543
Neural networks, in neuronal signaling regulation, 195, 195*f*
Neuraminidase inhibitors, 497, 497*f*, 547*f*, 557, 559–560, 559*f*, 564
Neuregulins, cardiac, 297
Neuroanatomy. *See* Nervous system, anatomy of
Neuroendocrine dysfunction, hypertension in, 378
Neurofibromatosis, gene therapy for, 763
Neurogenic diabetes insipidus, 410, 411*f*
Neurohormonal regulation
  in congestive heart failure, 394–395, 395*f*, 396*f*
  modulators of, for hypertension, 382
  of vascular tone, 322
  of volume, 301–304, 302*f*, 303*f*
Neurohypophysis
  hormones of, 410–411, 411*f*
  physiology of, 404
Neuroleptic(s). *See also* Antipsychotic drugs
  definition of, 171
Neuroleptic malignant syndrome, 173
Neuromuscular activity, of helminths, 598–599, 602
Neuromuscular blockade
  from aminoglycosides, 540
  as anesthetic adjuncts, 228
  depolarizing, 101, 102*t*
  nondepolarizing (competitive), 101, 102*t*
Neuromuscular junction, acetylcholine effects on, 94, 95*f*–97*f*
Neuron(s)
  afferent, in nociception, 122
  baseline tone of activity, 180
  cholinergic, 80*f*
  dopaminergic, 80*f*
  firing of
    abnormal. *See* Seizure(s)
    regulation of, 194–195, 195*f*, 196*f*
  GABAergic, in opioid abuse, 255, 256*f*
  nociceptive, 231
  noradrenergic, 80*f*
  organization of, 78–80
    local circuit, 78*f*, 79
    long tract, 78–79, 78*f*
    single-source divergent, 78*f*, 79–80, 79*t*, 80*f*
  postganglionic, 72, 73*f*, 74*f*
  preganglionic, 72, 73*f*, 74*f*
  primary afferent, excitation of, 230–232, 231*f*, 231*t*
  serotonergic, 80*f*
  spinal cord, 77–78
Neuropathic pain, 237, 237*f*
Neuropeptides, in neurotransmission, 80, 83–84, 232
Neurophysiology. *See* Blood–brain barrier; Neurotransmitters
Neurotoxins, 726–727, 730
Neurotransmission
  abnormal, in central nervous system. *See* Epilepsy; Seizure(s)
  adrenergic, 180–183
  dopaminergic. *See* Dopamine
  GABAergic, 139, 147–159
  glutamatergic, 139–147, 157
  serotonergic, 180–183
Neurotransmitters, 80–86. *See also specific neurotransmitters*
  action of, 66–69, 66*f*, 68*f*, 140, 140*f*
  amino acid. *See* Amino acid(s), as neurotransmitters; Gamma-aminobutyric acid; Glutamate
  biogenic, 80–83, 83*f*, 84*f*
  diffusely projecting systems of, 180
  excitatory. *See also* Glutamate
    action of, 140, 140*f*
  false, 113
  histamine as, 648*t*, 649
  inhibitory. *See also* Gamma-aminobutyric acid (GABA)
    action of, 140, 140*f*
  metabolism of, 68–69
  neuropeptides, 80, 83–84
  in single-source neuronal organization, 79–80, 79*t*, 80*f*
  structures of, 83*f*, 84*f*
  synthesis of, 81–82, 84*f*
  types of, 80, 81*f*, 82*t*
Neurotrophins, in pain transmission, 232
Neutral protamine Hagedorn (NPH) insulin, 466*t*, 467, 470
Neutropenia, drugs for, 662–663, 665
Neutrophils, 618–620, 619*f*, 620*t*, 656*t*, 657*f*, 659
Nevirapine, 555–556, 557*f*, 564
New Drug Application (NDA), 796–797, 797*f*
  Abbreviated, 798
New drug evaluation, 791–798
  approval phase of, 794–798
    clinical trials, 795–796, 795*t*
    fast track, 797
    FDA decision, 796–797, 797*f*
    for generic drugs, 797–798
    life cycle of, 792, 792*f*
    "not approved" applications, 796–797
    for orphan drugs, 797
    in other countries, 798
    preclinical evaluation, 749*f*, 753, 794–795, 794*f*
  case study of, 791–792
  ethics in, 793–794
  oversight of, 792–794, 792*f*, 793*t*
NFAT (nuclear factor of activated T cells) transcription factor, inhibitors of, 674, 674*f*, 680
NHE3 sodium/hydrogen ion exchanger, in volume regulation, 305, 305*f*
Niacin, for dyslipidemia, 370–371, 371*f*, 373
Nicorandil, in vascular tone regulation, 329, 332
Nicotine
  abuse of, 248*t*
  action of, 257, 257*f*
  administration of, for nicotine withdrawal, 259
Nicotinic acetylcholine receptors (nAChRs), 90–93, 91*f*, 92*t*, 93*f*, 94*f*
  agonists of, 101, 102*t*, 105
  antagonists of, 102, 105
  structure of, 8, 9*f*, 92–93, 93*f*
Nicotinic acid (niacin), for dyslipidemia, 370–371, 371*f*, 373
Nifedipine
  for arrhythmias, 281
  for coronary artery disease, 387
  for hypertension, 381
  solvent activation of, 785
  in vascular tone regulation, 327–328, 328*t*, 332
Nifurtimox, 596, 601
Nigrostriatal system, 76
  dopaminergic neurons in, 164
  neuronal organization of, 79
  physiology of, 166, 167*f*
Nitrates, 322–326
  for congestive heart failure, 396, 396*f*, 397*t*
  contraindications to, 326
  for coronary artery disease, 387, 388–389
  mechanism of action of, 322–324, 324*f*, 325*f*
  pharmacokinetics of, 324–325, 325*f*
  sildenafil interactions with, 326–327
  smooth muscle effects of, 326
  tolerance to, 324*f*, 325–326, 388–389
  toxicity of, treatment of, 742
  for vascular tone modulation, 331
Nitric acid, toxicity of, 735*t*
Nitric oxide (NO)
  donors of, in vascular tone regulation, 322
  in endothelial dysfunction, 385–386
  formation of, from nitrates, 322–324, 324*f*
  as neurotransmitter, 80, 83
  structure of, 83*f*
  in vascular tone regulation, 320, 321*f*
Nitric oxide synthase, endothelial isoform of, 320, 321*f*
Nitric oxide-releasing NSAIDs, 691
Nitrites, toxicity of, 735*t*
Nitro reduction, in drug metabolism, 47*t*
Nitrogen mustards, 524–527, 525*f*, 526*f*, 533
  as carcinogens, 727
  inhalation of, 721
  toxicity of, 736*t*
Nitroglycerin (NTG), 322–326, 331
  for angina pectoris, 318
  for congestive heart failure, 396, 396*f*

  - contraindications to, 326
  - for coronary artery disease, 388–389
  - mechanism of action of, 322–324, 324*f*, 325*f*
  - metabolism of, nitric oxide formation in, 320
  - pharmacokinetics of, 324–325, 325*f*
  - smooth muscle effects of, 326
  - structure of, 325*f*
  - tolerance to, 324*f*, 325–326
  - toxicity of, 736*t*
  - for unstable angina, 389
- Nitroprusside, 324, 324*f*, 325*f*, 331
- Nitroreductases, in *Entamoeba histolytica* metabolism, 595*f*
- Nitrosoureas, 525*f*, 526, 533
- Nitrous oxide, 227
  - analgesic index of, 207
  - blood/gas partition coefficient of, 214*f*, 215*f*, 220
  - delivery of, ventilation effects on, 216, 216*f*
  - MAC of, 207
  - oil/gas partition coefficient of, 210*f*, 220
  - pharmacology of, 220
  - properties of, 208*t*
  - recovery from, 219, 219*f*
  - structure of, 222*f*
  - tissue equilibration of, 214*f*
  - tissue/blood partition coefficients of, 213*t*
  - volume capacity for, 211*f*
- Nizatidine, 652, 688, 688*f*, 693
- NKCC2 (sodium-potassium-chloride cotransporter), in volume regulation, 306, 306*f*
- $N_M$ receptors, 93
- NMDA receptor(s)
  - in alcohol abuse, 257
  - in amyotrophic lateral sclerosis, 147
  - in central sensitization, 236, 236*f*
  - in hyperalgesia, 145, 146*f*
  - phencyclidine effects on, 259
  - physiology of, 141–142, 142*f*, 143*t*
  - receptor interactions of, 146*f*
  - in stroke, 145, 145*f*
- NMDA receptor antagonists, 241, 245
  - for hyperalgesia, 145
  - for stroke, 145
- $N_N$ receptors, 93
- Nociception
  - definition of, 122
  - overview of, 230, 230*f*
  - physiology of, 122–123, 123*f*–126*f*, 125*t*
- Nociceptive neuron(s), 231
- Nociceptive pain, 234
- No-effect dose, in preclinical testing, 794
- Non-anesthetics, with anesthetic chemical structure, 222
- Non-blinded clinical trials, 795
- Noncompetitive antagonists, 7, 21*f*, 22–23, 22*f*
  - spare receptor interactions with, 24–25, 25*f*
- Nondepolarizing (competitive) neuromuscular blockade, 101
- Nonimmobilizers, with anesthetic chemical structure, 222
- Nonpacemaker cells, in heart, 268, 272
- Nonprescription drugs, regulation of, 801
- Nonreceptor antagonist, 21, 21*f*, 23
- Nonreceptor antagonists, 23
- Nonreceptor tyrosine kinases. *See* Tyrosine kinase-associated receptors
- Nonsteroidal anti-inflammatory drugs (NSAIDs), 239–241, 240*f*, 638–640, 639*f*, 644
  - action of, 235–236
  - antiplatelet activity of, 345, 346*f*, 347*f*
  - for gout, 712, 715
  - gastropathy from, 638
  - nephrotoxicity of, 727
  - nitric oxide-releasing, 691
  - peptic ulcer disease from, 686–687, 687*f*
  - for peripheral sensitization, 235–236
  - for rheumatoid arthritis, 234
  - selection of, 640
- Nontransmural myocardial infarction, 386
- Noradrenergic neurons, 80*f*
- Norepinephrine (NE), 80, 162
  - action of, 112, 183
  - degradation of, 181*f*, 182
  - depressed neurotransmission of, in depression, 184–185, 186*f*
  - inotropic effects of, 295*t*, 296, 298
  - metabolism of, 110, 110*f*
  - monoamine oxidase inhibitor effects on, 188–189
  - neurons containing, 180
  - in pain transmission regulation, 232, 233, 234*f*
  - receptors for, 112, 183, 183*t*
  - regulation of, 181–182, 181*f*
  - release of, 108*f*, 109, 109*f*
  - reuptake of, 109*f*, 110
    - inhibitors of, 114
    - tricyclic antidepressant effects on, 186–187, 187*f*
  - for shock, 114
  - storage of, 108*f*, 109
  - structure of, 83*f*
  - sympathomimetic effects on, 113, 113*f*
  - synthesis of, 81–82, 84*f*, 108–109, 108*f*, 162–163, 162*f*, 181, 181*f*
  - in vascular tone regulation, 322
- Norepinephrine autoreceptors, 185, 186*f*
- Norepinephrine transporter (NET), 109*f*, 110, 112, 113, 181*f*, 182
  - blocking of, by stimulant drugs, 257
  - inhibition of, 187*f*
- Norethindrone, 452, 453, 456
- Norgestimate, 452, 456
- Norgestrel, 453, 456
- Norplant contraceptive system, 784
- Nortriptyline, 191, 241, 245
- Novel erythropoiesis stimulating protein (darbepoietin, NESP), 661–662, 665
- NPH (neutral protamine Hagedorn) insulin, 466*t*, 467, 470
- NR subunits, of NMDA receptors, 143*t*
- NSAIDs. *See* Nonsteroidal anti-inflammatory drugs (NSAIDs)
- NTG. *See* Nitroglycerin (NTG)
- N-type voltage-gated calcium ion channels, in pain transmission, 232, 233*f*
- Nuclear factor of activated T cells (NFAT) transcription factor, inhibitors of, 674, 674*f*, 680
- Nuclear magnetic resonance
  - in drug development, 754
  - in drug structural analysis, 751–752
- Nucleic acids. *See also* DNA; RNA
  - synthesis of, 508–509
- Nucleophiles, 520, 521*f*, 722
- Nucleosides
  - analogues of. *See* Antiviral drugs, genome replication inhibitors
  - definition of, 506
- Nucleotide(s)
  - biochemistry of, 506–509
  - definition of, 506
  - nucleic acid synthesis from, 508–509
  - structures of, 506, 507*t*
  - synthesis of, 506–508, 506*f*, 507*t*, 508*f*–511*f*
    - inhibitors of. *See* Antimetabolites
- Nucleus accumbens, 76
  - dopamine receptors in, 164, 165*f*
  - in reward pathway, 251–252, 257*f*
- Nucleus basalis, 80*f*
- Nucleus basalis of Meynert, acetylcholine effects on, 96
- Nutritional rickets, 480, 480*t*, 486
- Nystatin, 583, 585

**O**

- Obesity
  - diabetes mellitus in, 464, 465
  - half-life and, 39
- Observer bias, in clinical trials, 795
- Occipital lobe, 75, 76*f*
- Occupational Safety and Health Administration (OSHA), 743
- Occupational safety, toxic exposures in, 719
- Octopamine, 113
- Octreotide
  - for hyperinsulinemia, 468–469, 471, 472*f*
  - for somatotroph adenoma, 407, 413
- Oculomotor nerve, parasympathetic fibers in, 73, 74*f*
- "Off-label" drug use, 799
- Ofloxacin, 529, 534
- Ohm's law, 60, 60*f*
- Oil/gas partition coefficient, of general anesthetics, 207, 208*t*, 209*b*, 210*f*, 220
- Oil–water partition coefficient, blood–brain barrier and, 85, 85*f*
- OKT3 monoclonal antibody, 677, 681, 778
- Olanzapine, 174–175, 178
- Olfactory bulb, dopaminergic neurons in, 166*f*
- Olfactory tubercle, dopamine receptors in, 164, 165*f*
- Omalizumab, for asthma, 703, 707
- Omeprazole, for peptic ulcer disease, 689, 690*b*, 693
- *Onchocerca volvulus*
  - drugs for, 598–599, 602
  - physiology of, 597–598, 597*f*
- Oncotic pressure, in volume regulation, 300, 301*f*
- One-pot synthesis, in drug development, 755
- Open state, of sodium ion channels, 194, 195*f*
- Opioid(s), 84
  - abuse of, 248*t*, 255, 256*f*
    - detoxification in, 259–260, 260*f*
    - pharmacologic treatment of, 261–263
  - in pain transmission regulation, 232, 234*f*
  - receptors for
    - agonists of, 238–239, 238*f*, 244, 263
    - antagonists of, 238, 244, 263. *See also* Naloxone; Naltrexone
  - toxicity of, 741
- Oprelvekin, 661*f*, 663, 666
- Opsonins, in phagocytosis, 624–625
- Oral contraceptives, 452–453, 456
  - hepatotoxicity of, 726
  - toxicity of, 736*t*
- Oral delivery, 30–31, 31*t*, 782
  - of anticoagulants, 350–353, 351*f*, 352*t*
  - of calcium, 486, 489
  - of combination therapy, for diabetes mellitus, 468, 469*t*
  - of cortisol, 432
  - formulations for, 755–756, 756*t*
  - of phosphate, 486–487
- Organ dysfunction, in toxic exposures, 722
- Organic arsenic compounds, hepatotoxicity of, 726
- Organic nitrates
  - for congestive heart failure, 396, 396*f*, 397*t*
  - for coronary artery disease, 388–389

- Organic nitrates (*contd.*)
  - for vascular tone modulation, 322–326, 324*f*, 325*f*, 331
- Organification, of thyroglobulin, 416
  - inhibitors of, 420–421, 420*f*, 423
- Organogenesis, teratogen effects on, 728
- Organophosphate nerve gases, 724, 725*f*, 742
- Oropharyngeal candidiasis, from glucocorticoid therapy, 433
- Orotate, in ribonucleotide synthesis, 507–508, 508*f*, 510*f*
- Orphan Drug Amendments of 1983, 793*t*, 797
- Orphan drugs, 797
- Orthostatic hypotension, from tricyclic antidepressants, 187
- Oseltamivir, 547*f*, 559–560, 559*f*, 564
- OSHA (Occupational Safety and Health Administration), 743
- Osmosis, in polymer-based drug delivery, 784*f*, 785
- Osmotic diuretics, 312, 316
- Osmotic forces, in edema, 307
- Osmotic pressure, in bacterial cell wall maintenance, 566
- OspA vaccine, 779
- Osteitis fibrosa cystica, 481, 483, 483*f*
- Osteoblasts, 474, 474*f*, 475, 476*f*
  - impairment of, in osteoporosis, 482, 482*f*
  - parathyroid hormone effects on, 476–477
- Osteoclasts, 474, 474*f*, 475, 476*f*
  - calcitonin receptors on, 479
  - function of, 659
  - increased activity of, in osteoporosis, 482, 482*f*
  - parathyroid hormone effects on, 476–477
- Osteocytes, 474, 474*f*
  - impairment of, in osteoporosis, 482, 482*f*
- Osteoid, in remodeling, 475, 476*f*
- Osteomalacia, 479–480
- Osteoporosis, 480*t*, 481–482, 482*f*
  - calcium wasting in, thiazides for, 313
  - case study of, 473–474
  - from glucocorticoid therapy, 432
  - versus osteomalacia, 479
  - vitamin D for, 486
- Ototoxicity
  - of aminoglycosides, 539–540
  - of loop diuretics, 312
- Ouabain, 291
- Outer medullary collecting duct, in volume regulation, 306–307, 307*f*
- Outer membrane, of bacterial cell wall, 566, 566*f*
- Ovarian hyperstimulation syndrome, 410
- Ovarian hypothesis, of polycystic ovarian syndrome, 448
- Ovary
  - hormone action on, 445, 445*f*
  - polycystic disease of, 437, 441–442, 447–448
- Overdrive suppression, in heart rhythm, 271
- Overflow model, of sodium retention, 308–309, 309*f*
- Ovulation, 445, 446*f*
  - induction of, clomiphene for, 451, 455
  - inhibition of, contraceptives for, 452–453, 456
- Oxacillin, 573, 577
- Oxazolidinones, 537*f*, 539*t*, 542, 544
- Oxicams, as NSAIDs, 639*f*, 640, 644
- Oxidants, macromolecular damage from, 722
- Oxidation, in drug metabolism, 35, 50*f*, 46*t*
- Oxidative phosphorylation, lack of, of *Entamoeba histolytica*, 594*f*
- Oxycodone, 239, 244, 255, 256
- Oxygen
  - deficiency of. *See* Hypoxia; Ischemia
  - toxicity of, 726
- Oxygen demand, myocardial
  - determinants of, 318, 319*f*, 319*t*
  - reduction of, 387–389, 387*t*
- Oxyhemoglobin, vs. carboxyhemoglobin, 726*f*
- Oxymetazoline, 115, 119
- Oxypurinol, 713, 713*f*
- Oxytocin, 410–411
- Ozone, toxicity of, 726

**P**

- P (peptidyl) site, of ribosome, 537, 537*f*
- p53 tumor suppressor gene, 728
  - protein-based therapeutics targeting, 760
- P wave, in electrocardiogram, 271*b*, 272*f*
- Pacemakers, in heart, 268. *See also* Sinoatrial node
  - latent, 270–271
  - native, 270
- Package inserts, 798, 799*f*
- Packaging cell line, in gene therapy, 767
- Paclitaxel, 531, 534, 748–749, 750*t*
- Paget's disease, 482
- Pain
  - chemical mediators in, 623*t*
  - chest, in myocardial ischemia, 318. *See also* Angina (pectoris)
  - as classic sign of inflammation, 617
  - definition of, 230
  - first, 122, 125*f*
  - in gout, 709, 711–712, 711*t*
  - hypersensitivity to (hyperalgesia), 145, 146*f*, 235
  - neuropathic, 237, 237*f*
  - nociceptive, 234
  - pathophysiology of, 234–237
    - central sensitization, 236–237, 236*f*, 237*f*
    - clinical pain, 234–235
    - in migraine, 237
    - peripheral sensitization, 235–236, 235*f*
  - perception of, 123, 126*f*
  - physiology of, 122–123, 123*f*–126*f*, 125*t*, 230–233
    - acetylcholine in, 95–96
    - conduction to spinal cord, 232
    - overview of, 230, 230*f*
    - regulation in spinal cord, 232–233, 234*f*
    - sensory transduction, 230–232, 231*f*, 231*t*
    - transmission in dorsal horn, 232, 233*f*
  - second, 122–123, 125*f*
  - spontaneous, 234
  - treatment of, 234. *See also* Analgesia
- Palivizumab, 760, 778
- $P_{alv}$ (alveolar partial pressure), of anesthetics, 207, 210–217, 214*f*–216*f*
- Pamidronate, 484, 488
- Pancreatic enzymes
  - for cystic fibrosis, 759
  - therapy with, 773
- Pancreatic hormones. *See* Diabetes mellitus; Glucagon; Insulin; Somatostatin
- Pancreatic polypeptide, 458
- Pancuronium, 105
  - as anesthetic adjunct, 228
  - clinical applications of, 101
  - with general anesthesia, 221
- Pancytopenia, 658
- Pantoprazole, for peptic ulcer disease, 689, 690*b*, 693
- Papain, 759, 777
- Para-aminobenzoic acid
  - analogues of, 509–511, 510*f*, 511*f*
  - in folate synthesis, 511*f*
  - as procaine metabolite, 133
- Para-aminophenols, 240. *See also* Acetaminophen
- Paracetamol. *See* Acetaminophen
- Parafollicular C cells, 415
- Parallel synthesis, in drug discovery, 751
- Paramyxovirus infections, Paget's disease in, 482
- Parasites
  - drug targets in, 498
  - helminths, 597–599, 597*f*, 602
  - luminal protozoa, 593–597, 594*f*, 595*f*, 601
  - malarial plasmodia. *See* Malaria
- Parasympathetic nervous system
  - anatomy of, 73, 73*f*, 74*f*
  - in automaticity regulation, 272
  - neurotransmitters in, 81*f*
  - in vascular tone regulation, 322
- Parathion, toxicity of, 724, 725*f*, 736*t*
- Parathyroid gland
  - adenoma of, 481
  - hyperplasia of, 481
- Parathyroid hormone (PTH), 752*t*
  - abnormal secretion of, in kidney failure, 483, 483*f*
  - for bone disorders, 485–486, 489
  - in calcium homeostasis, 475, 475*f*, 476–477, 477*t*, 478*f*
  - deficiency of (hypoparathyroidism), 480*t*, 481
  - excess of (hyperparathyroidism), 312, 432, 480–481
  - PTH(1-34) fragment of, 485–486
  - receptor for, 481
  - recombinant, 775
- Paravertebral ganglia, 73*f*, 74*f*
- Parecoxib, 641
- Parenteral drug administration, 31–32, 31*t*
  - formulations for, 755–756, 756*t*
- Parietal cells, gastric acid secretion from, 684, 685*f*
- Parietal lobe, 75, 76*f*
- Park peptide
  - beta-lactam interactions with, 572
  - in murein synthesis, 567, 569*b*
- Parkinson's disease, 166–170
  - case study of, 161–162
  - drugs for, 168–170, 168*f*, 176
  - glutamate receptors in, 147
  - levodopa for, 76
  - nigrostriatal pathways and, 166, 167*f*
  - pathophysiology of, 166–167, 167*f*
- Paromomycin, 539, 543, 596
- Paroxetine, 187, 191, 241
- Paroxysmal depolarizing shift, in partial seizures, 195, 199
- Paroxysmal supraventricular tachycardia (PSVT), 276*b*
- Pars compacta, dopaminergic neurons in, 164, 166
- $P_{art}$ (arterial partial pressure), of anesthetics, 213, 214, 215*f*, 216
- Partial agonists, 23–24, 23*f*
  - definition of, 7, 20
  - opioid, 239
- Partial antagonists. *See* Partial agonists
- Partial pressure, of anesthetics
  - alveolar, 207, 210–217, 214*f*–216*f*
  - arterial, 213, 214, 215*f*, 216
  - brain, 213
  - CNS, 206–207, 213, 216–218
  - vs. concentration, 207*b*
  - inspired, 207, 210–212, 214–218, 214*f*, 216*f*, 218*f*
  - in mixed venous return, 215–216, 226
  - tissue, 212–213, 213*t*
  - VRG (vessel rich group), 210, 211*f*, 213*t*, 214, 214*f*, 219
- Partial seizures
  - definition of, 195, 196*t*

  drugs for, 198–201, 203–204
  pathophysiology of, 195–196, 197f
  with secondary generalization, 196t
Partition coefficients, 754
  of general anesthetics
    blood/gas, 214–215, 214f, 215f, 219, 219f
    oil/gas, 207, 208t, 209b, 210f, 220
    tissue/blood, 212–213, 213t, 214f
Passive targeting, of tissues, for drug delivery, 786
Patch, transdermal, 31t, 32, 454
Patents, drug, 801
Pattern recognition, of infectious agents, 620
PBPs (penicillin-binding proteins, transpeptidases), 567, 568f, 569, 570f
$P_{brain}$ (brain partial pressure), of anesthetics, 213
$P_{CNS}$ (CNS partial pressure), for anesthetics, 206–207, 213, 216–218
PCOS (polycystic ovarian syndrome), 437, 441–442, 447–448
PCP (phencyclidine), 248t, 259
Peak concentration, vs. absorption rate, 32, 32f
Pediatric patients, anesthesia induction in, 216, 217f
Pedunculopontine nucleus, 79–80, 79t, 80f
Pegylated recombinant human megakaryocyte growth and development factor, 663
Pemphigus vulgaris, 670t
Penciclovir, 551f, 552–553, 562
Penicillamine, 741, 741f
Penicillin(s), 565, 568f, 572–575, 577
  aminoglycoside synergism with, 607
  antipseudomonal, 575
  antistaphylococcal, 574
  beta-lactamase inhibitors with, 607. *See also specific combinations*
  case study of, 565–566
  discovery of, 565, 748
  GABA receptor binding site for, 148f
  seizures induced by, 194
  structures of, 572, 572f
  in transpeptidase inhibition, 569, 570f
Penicillin G, 573, 574, 577, 750t
Penicillin V, 574, 577
Penicillin-binding proteins (PBPs, transpeptidases), 567, 568f, 569, 570f
Pentadecacatechol
  hypersensitivity to, 724
  toxicity of, 736t
Pentamidine, 596, 601
Pentobarbital, 159
  action of, 150, 151t
  clinical applications of, 152t
  vs. phenobarbital, 151t
  structure of, 222f
Pentostatin, 514, 514f, 517
Pepsin, in peptic ulcer disease, 687
Peptic ulcer disease, 684–693
  case study of, 683–684
  drugs for, 688–694
    acid secretion inhibitors, 688–690, 688f, 689f, 690b, 693
    antacids, 690, 693
    antibiotics, 691
    anticholinergic, 690, 694
    H2 receptor antagonists, 652, 653f, 654
    mucosal defense promoters, 690–691, 694
    prostaglandins, 691, 694
    proton pump inhibitors, 688–690, 689f, 690b
    sites of action of, 692f
  epidemiology of, 683
  gastric acid secretion physiology and, 684–685, 685f
  lifestyle modifications for, 691
  pathophysiology of, 685–687, 686f, 686t, 687f
Peptides, in drug design, 752–753, 752t
Peptidoglycans, in cell wall. *See* Murein
Peptidomimetics, in drug design, 752–753
Peptidyl (P) site, of ribosome, 537, 537f
Peptidyl transferase, in translation, 537f, 538
Pepto Bismol, 690–691, 694
Perceived volume depletion, in congestive heart failure, 307–308, 308f
Perchlorate, for hyperthyroidism, 420, 420f, 423
Perchloroethylene (tetrachloroethylene), toxicity of, 721, 737t
Perfusion
  in inhaled anesthetic distribution, 210
  regulation of, vascular tone in. *See* Vascular tone
Perfusion-limited inhaled anesthetics, 214, 214f, 216, 216f
Pergolide
  for Parkinson's disease, 169
  for prolactinoma, 408, 413
Periaqueductal gray, 77
Perindopril, 315
Peripheral nerve blockade, 132
Peripheral nervous system
  anatomy of, 72–75, 73f, 74f
  neurotransmitters in, 80, 81f
Peripheral neuropathy
  from taxanes, 531
  from vincristine, 530
Peripheral sensitization, 235–236, 235f
Peripheral tolerance, of immune system, 669
Periplasmic space, bacterial cell wall synthesis in, 567, 568f
Periventricular nuclei, dopaminergic neurons in, 166
Permanent plug, in hemostasis, 336
Permeases, in fungal membranes, flucytosine effects on, 581f, 583, 585
Peroxisome proliferator response elements, fibrate activation of, 370, 370f
Peroxisome proliferator-activated receptor α
  celecoxib effects on, 641
  fibrate activation of, 369–370, 370f
Peroxisome proliferator-activated receptor γ (PPARγ), inhibitors of, 467–468
Peroxynitrite, in nitrate tolerance, 326
Perphenazine, for schizophrenia, 177
Personality differences, in addiction, 255
Pertechnetate, for hyperthyroidism, 420, 420f, 423
Pesticides
  ingestion of, 720
  toxicity of, 724, 725f
Pet dander, hypersensitivity to, 723, 736t
Petit mal (absence seizures)
  definition of, 196t
  drugs for, 201, 203–204
  pathophysiology of, 197, 199f
PFA (phosphonoformic acid, foscarnet), 555, 557f, 563
PfCRT transporter, in heme metabolism, 589, 589f, 591
PfEMP, in malaria, 589
PFOR (pyruvate:ferredoxin oxidoreductase), in *Entamoeba histolytica* metabolism, 594–595, 595f
P-glycoprotein, in drug resistance, 529
pH, local anesthetic action and, 124–125
pH trapping, 29, 29f
Phagocytosis, 618–619, 621f, 624–625
Pharmacodynamic(s), 17–26
  case study of, 17
  definition of, 7, 17
  dose-response relationships, 19–20, 19f, 20f
  drug-receptor binding. *See* Binding, drug-receptor
  drug-receptor interactions, 20–25, 21f–25f
  therapeutic concepts, 25
Pharmacodynamic tolerance, 250–251, 252f, 325
Pharmacogenomics, 803–809
  case study of, 803
  of drug metabolism, 52
  of enzymes of drug metabolism, 806–808, 807f, 807t, 808f
  genomic variations in populations, 803–804, 804f
  of idiosyncratic effects, 808–809, 809t
  of target proteins and pathways, 805–806, 805t
Pharmacokinetic(s), 27–43. *See also specific drugs*
  absorption, 27, 28f, 30–32, 30f, 31t, 32f
  case study of, 27–28
  clearance. *See* Clearance
  distribution, 27, 28f, 33–35, 33t, 34f–37f
  in drug design, 754
  excretion, 27, 28f, 35–38, 37f
  half-life, 39, 39t, 40b
  metabolism. *See* Metabolism, drug
  overview of, 27, 28f
  physiologic barriers and, 28–29, 29f
  relationships in, 43, 43t
  therapeutic dosing, 40–43, 41f, 42f
Pharmacokinetic tolerance, 250, 251f
Pharmacologic treatment, for addiction, 259–262
Pharmacology review, of new drugs, 795, 796, 797f
Phase(s)
  of cardiac action potentials, 268–270, 269f, 270t, 271f
  in seizures, tonic and clonic, 197
Phase I reactions, in drug metabolism, 35, 45–49, 46t–47t, 50f, 806, 807t
Phase I studies, of new drugs, 795, 795t
Phase II reactions, in drug metabolism, 35, 45–47, 48t, 49, 51f, 806, 807t
Phase II studies, of new drugs, 795–796, 795t
Phase III studies, of new drugs, 795t, 796
Phase IV studies, of new drugs, 799, 800t
Phasic firing, in GABAergic transmission, 148
Phasic inhibition, of sodium ion channels, 128, 130f
Phencyclidine (PCP)
  abuse of, 248t
  action of, 259
  psychotic effects of, 171
Phenelzine, 114, 118, 188, 191
Phenobarbital, 159
  action of, 150, 151f, 151t
  as carcinogen promoter, 728
  clinical applications of, 152, 152t
  metabolism of, 152t
  overdose of, 139–140
  vs. pentobarbital, 151t
  for seizures, 202
Phenothiazines, 177, 652f
  for schizophrenia, 171
  structures of, 173f
Phenoxybenzamine, 116, 119, 381
Phentolamine, 116, 119, 381
Phenylalanine, in tyrosine synthesis, 162
Phenylalkylamines, in vascular tone regulation, 327
Phenylephrine, 115, 119
Phenylethanolamine-N-methyltransferase, in catecholamine synthesis, 108f, 109, 162f, 163
Phenylethylamines, abuse of, 248t
Phenylheptylamines, 239

Phenylpiperidine, 239
Phenylpropanolamine, 113, 118
Phenytoin, 198–201, 200f, 203
Philadelphia chromosome, in chronic myeloid leukemia, 4
Phosfomycin, 568f, 570–571, 577
Phosgene, toxicity of, 726
Phosphate
  homeostasis of, 476, 477t
  oral, for hypercalcemia, 486–487
Phosphatidylserine, in coagulation, 338, 341f
Phosphodiesterase inhibitors
  antiplatelet activity of, 345, 347f, 348
  for congestive heart failure, 398
  inotropic effects of, 296–297
  in vascular tone regulation, 322, 326–327, 331
Phosphoenolpyruvate, in murein synthesis, 567, 568f
Phospholamban, in cardiac contractility, 288, 290f
Phospholipase(s), inhibitors of, 638, 645
Phospholipase $A_2$
  in arachidonic acid synthesis, 628, 628f
  in platelet activation, 336, 339f
Phospholipase C
  in drug-receptor interactions, 9, 11f
  in peripheral sensitization, 235, 235f
  in platelet activation, 339f
  in platelet aggregation, 338, 339f
Phospholipid(s), in arachidonic acid synthesis, 628, 628f
Phospholipid-based protein-protein complex, 338, 341f
Phosphonoformic acid (foscarnet, PFA), 555, 557f, 563
Phosphoribosylpyrophosphate (PRPP), in purine synthesis, 710, 710f
Phosphorylation, of proteins, 10, 14, 15f
Physical dependence, on opioids, 238
Physical (physiologic) dependence
  definition of, 249–250
  mechanisms of, 251
Physical properties, evaluation of, in drug development, 754
Physiologic antagonist, 21, 21f, 23
Physiologic barriers, 28–29, 29f
Physiologic tolerance, to nitrates, 325
Physiology, of nervous system. *See* Blood–brain barrier; Neurotransmitters
Physostigmine, 104
  action of, 98, 99
  for belladonna poisoning, 742
  clinical applications of, 100
  structure of, 99, 99f
  toxins resembling, 724
$P_I$ (inspired partial pressure), of anesthetics, 207, 210–212, 214–218, 214f, 216f, 218f
Picrotoxin, 148f, 149t, 150
Pilocarpine, 104
  action of, 83, 101
  structure of, 100f
Pimozide, 177
Pinacidil, in vascular tone regulation, 329, 332
Pindolol, 116, 117t, 119
  for arrhythmias, 279
  as partial agonist, 24
  pharmacodynamics of, 387t
Pinworms, drugs for, 598–599, 602
Pioglitazone, for diabetes mellitus, 460f, 467–468, 471
Piperacillin, 573, 575, 577
Piperazine, 598–599, 602
Pirbuterol, for asthma, 699, 706
Pirenzepine, 105
  action of, 97
  clinical applications of, 101
Pirmagrel, 642, 646
Piroxicam, 240, 244, 639f, 640, 644
Pituitary gland
  anterior
    in hypothalamic axes, 406–410, 407f, 409f
    physiology of, 404, 404f, 405t
  dopamine receptors in, 164, 165f
  dysfunction of, 405–406
  physiology of, 404–406, 404f, 405t, 406f
  posterior
    hormones of, 410–411, 411f
    physiology of, 404
pKa, of local anesthetics, 125
$pK_a$, 29, 29f
Plasma cells, 657f
Plasma exchange, for toxin elimination, 740
Plasma membrane. *See* Cell (plasma) membrane
Plasma protein binding, 34, 34f
Plasma volume regulation. *See* Volume regulation
Plasmepsins, in heme metabolism, 589, 589f
Plasmids, in drug resistance, 501, 573
Plasmin, in hemostasis regulation, 342, 343f
Plasminogen, in hemostasis regulation, 343f
Plasminogen activator inhibitor, in hemostasis regulation, 342–343, 343f
Plasmodia. *See* Malaria
Plate, for high-throughput drug screening, 751
Platelet(s), 656, 656t, 657f
  activation of, 336, 338, 338f
  adhesion of, 336, 337f
  aggregation of, 337f, 338, 339f, 340f
  drugs inhibiting. *See* Antiplatelet agents
  function of, 336, 337f, 338, 339f, 340f
  granule release from, 336, 337f, 338, 338f
  production of, 661, 661f
    inducers of, 663, 666
  prostaglandin effects on, 630f, 631, 632f
  structure of, 336
Platelet-activating factor, in asthma, 697f, 698
Platelet-derived growth factor, recombinant, 775
Platinum compounds, 527, 527f, 528f, 533, 610t
  hypersensitivity to, 723, 736t
Pleiotropic effects
  in hematopoiesis, 657–658
  of statins, 368
Plug, hemostatic, 336, 338
Pluripotent hematopoietic stem cells, 656, 657f
$P_{MVR}$ (mixed venous return partial pressure), 215–216, 226
Pneumoconioses, in dust exposures, 726
*Pneumocystis carinii* pneumonia, drugs for, 596
Pneumonitis, from amiodarone, 280
Podagra, 711
Poison ivy, 724
Poisons. *See* Toxic exposures; Toxicity; *specific poisons*
Pollutants, drug metabolism and, 53
Polyanhydride polymers, in drug delivery, 785, 785f
Polyclonal antibodies, for immunosuppression, 676–677, 681
Polycyclic aromatic hydrocarbons, as carcinogens, 727, 727f
Polycystic ovarian syndrome, 437, 441–442, 447–448
Polyenes, 581f, 582–583, 585
Polyethylene glycol polymers, in drug delivery, 894–785, 785f
Polygenic hypercholesterolemia, 365, 365t
Polymerase(s), DNA, inhibitors of. *See* Antiviral drugs, genome replication inhibitors
Polymerase inhibitors, for viral infections, 497–498, 497f
Polymer-based drug delivery systems, 783–786, 784f, 785f
Polymerization, in bacterial cell wall synthesis, 567, 568f
  inhibitors of, 572, 577
Polymicrobial infections, combination therapy for, 607–608
Polymorphism, 804, 804f
  in drug targets, 805–806, 805t
  in enzymes of drug metabolism, 806–808, 807f, 807t, 808f
  idiosyncratic effects related to, 808–809, 809t
Polymorphonuclear leukocytes, in fungal defense, 580
Polythiazide, 316
POMC (proopiomelanocortin), 408, 428
Pons, anatomy of, 75, 75f, 77
Pores
  in bacterial outer membrane, 566, 566f
  in fungal cell membrane, amphotericin effects on, 582–583
Porins, of bacterial outer membrane, 566, 566f
Portal circulation, in drug metabolism, 47, 49f
Posaconazole, 583
Positive inotropes, definition of, 285
Positive symptoms, in schizophrenia, 170
Posterior horn, of spinal cord, 75
Posterior pituitary gland, physiology of, 404
Postganglionic neurons, 72, 73f, 74f
Postictal period, 197
Postmarketing surveillance, after drug approval, 797
Postmenopausal osteoporosis, 481–482, 482f
Postsynaptic potentials
  excitatory, 67, 94–95, 97f
  inhibitory, 67, 94–95, 147–148
Postsynaptic receptors, 68
Potassium, for arrhythmias, 282
Potassium current
  in cardiac action potential, 268, 269f, 270, 270t, 271f
  hyperpolarizing, 279, 280f
  in myocardial ischemia, 278
Potassium hydroxide, macromolecular damage from, 722
Potassium ion
  in adrenocortical hormone regulation, 426, 426f
  contribution to resting potential, 63f
  loading of, aldosterone synthesis in, 436
  Nernst equilibrium potential for, 62, 62t
Potassium ion channel(s)
  in action potential, 63–66, 64f, 65f
  in AMPA receptor activation, 142, 143t
  blockers of
    for arrhythmias, 279–281, 280f, 281t
  in cardiac function, 268, 269f, 270, 270t, 271f
  in dopamine release, 164
  in $GABA_B$ receptor activation, 148–149, 149f
  in kainate receptor activation, 142, 143t
  metabotropic, in vascular tone regulation, 328–329
  neuronal firing and, 194–195, 194f
  neurotransmitter effects on, 140, 140f
  in nicotinic transmission, 92–93, 93f, 94f
  nitric oxide activation of, 320, 321f
  openers of
    action of, 323f
    for hypertension, 381
    in vascular tone regulation, 322, 328–329, 332
  pharmacology of, 66
  in volume regulation, 306, 306f
Potassium ion/ATP channel
  inhibitors of, 467–469
  in insulin secretion, 459, 461f

Potassium-sparing (collecting duct) diuretics, 313–314, 316, 380
Potency ($EC_{50}$), of drugs, 18, 18*f*, 40
  anesthetics, 223, 223*f*
  antagonist effects on, 22*f*
  spare receptor effects on, 24–25, 24*f*
Potentiation, of glutamate-gated chloride channels, in ivermectin action, 598
PP cells, pancreatic polypeptide secretion from, 458
PPARγ (peroxisome proliferator-activated receptor γ)
  celecoxib effects on, 641
  inhibitors of, 467–468
PPARα (peroxisome proliferator-activated receptor α), fibrate activation of, 369–370, 370*f*
PPREs (peroxisome proliferator response elements), fibrate activation of, 370, 370*f*
PR interval, in electrocardiogram, 271*b*, 272*f*
Pralidoxime, 99
  for organophosphate poisoning, 742
  toxicity of, 725*f*
Pramipexole, for Parkinson's disease, 169, 176
Pravastatin, 22, 369, 373
Praziquantel, 599, 602
Prazosin, 116, 119, 329, 333, 381
Precarcinogens, 727, 727*f*
Preclinical evaluation, of new drugs, 749*f*, 753, 794–795, 794*f*
Prednisolone, 439
  potency of, 430
  structure of, 431*f*
Prednisone, 439
  for cancer, 610*t*
  for inflammation, 638
  structure of, 431*f*
Preganglionic neurons, 72, 73, 73*f*, 74*f*
Pregnancy
  drug use in, 447*b*
  teratogenesis in, 447*b*, 722, 728
Pregnenolone
  in adrenocortical hormone synthesis, 426, 427*f*
  in reproductive hormone synthesis, 443*f*
Preload
  definition of, 286
  reduction of, in congestive heart failure, 395–396
  as stroke volume determinant, 392, 393*f*
Preproendothelin, 321, 321*f*
Preproinsulin, 459, 461*f*
Prevertebral ganglia, 72, 73*f*, 74*f*
Prices, drug, regulation of, 800–801
Prilocaine, 133, 135
Primaquine, 593, 600, 724, 736*t*
Primary afferent neurons, excitation of, 230–232, 231*f*, 231*t*
Primary (clinical) endpoints, 796
Primary generalized seizures
  definition of, 196*t*
  pathophysiology of, 197, 197*f*, 199*f*
Primary hemostasis, 336, 337*f*–340*f*, 338
Primary hemostatic plug, 336, 338
Primary hyperaldosteronism, 436
Primary hyperparathyroidism, 480–481, 480*t*
Primary (essential) hypertension, 377
Primary structure, of proteins, 4, 4*f*
Primase, 536
Principal cells, in volume regulation, 306–307, 307*f*
Probenecid, for gout, 710*f*, 714, 715
Procainamide
  for arrhythmias, 277, 283
  autoimmune disease from, 724
  toxicity of, 736*t*
Procaine, 121, 133, 135
  structure of, 126*f*
  volume of distribution of, 131
Procarbazine, 526, 533
Process patents, 801
Procoagulant factors, in hemostasis regulation, 340
Prodrugs, 35
  definition of, 45, 51
  toxification of, 721
Producer cell line, in gene therapy, 767
Proenzymes, in coagulation, 338
Progesterone
  in contraceptives, 452–453, 456
  inhibitors of, 449*f*
  in menstrual cycle, 445–446, 446*f*
  receptor for, antagonists of, 449*f*, 452, 456
  synthesis of, 442, 443*f*
Progestins
  actions of, 442
  in contraceptives, 452–453, 456
  implantable, 784
  synthesis of, 442, 443*f*
Progression, in carcinogenesis, 727
Proguanil, 593
Proinsulin, 459, 461*f*
Prolactin, 404
  in hypothalamic-pituitary-prolactin axis, 407–408
  inhibition of, 173, 408, 413
Prolactinoma, 408, 448
Proliferation, in carcinogenesis, 499, 609
Proliferative phase, of menstrual cycle, 445, 446*f*
Promethazine, 652*f*
Promotion, in carcinogenesis, 727, 728
Prontosil, 505–506
Proopiomelanocortin (POMC), 408, 428
Propafenone, for arrhythmias, 278, 283
Prophylactic chemotherapeutics, 503
Propionic acid derivatives, 240, 639*f*, 640, 644
Propofol, 227
  clinical applications of, 151
  pharmacology of, 221
  structure of, 222*f*
Propoxyphene, 239, 244
Propranolol, 116, 117*t*, 119
  for arrhythmias, 279, 283
  for hypertension, 380
  pharmacodynamics of, 387*t*
  for vascular tone regulation, 333
Propylthiouracil, for hyperthyroidism, 420*f*, 421, 423
Prostacyclin
  actions of, 631, 632*f*
  in hemostasis, 337*f*, 341
  synthesis of, 345, 346*f*, 628*f*, 630*f*, 631
Prostaglandin(s)
  actions of, 629, 630*f*, 631, 631*t*
  analogues of, for inflammation, 645
  degradation of, 633
  in gastric acid secretion regulation, 684
  inhibitors of. *See* Nonsteroidal anti-inflammatory drugs (NSAIDs)
  in nociception, 123*f*
  for peptic ulcer disease, 691, 694
  synthesis of, 345, 346*f*, 628*f*–630*f*, 629, 631, 631*t*
Prostaglandin H synthases. *See* Cyclooxygenases (COXs)
Prostanoid receptor mimetics, 641, 645
Prostanoid structure, 629, 629*f*
Prostate
  benign hyperplasia of, 116, 448
  cancer of, 448
Protamine, for heparin reversal, 355
Protease(s), therapy with, 773
Protease inhibitors, for HIV, 497, 497*f*, 547*f*, 556–557, 558*f*, 559*b*, 564, 747–748, 748*f*
Proteasome inhibitors, for cancer, 613
Protective factors, of gastric mucosa, 685, 686*t*
  promotion of, 690–691, 694
Protein(s)
  aggregation of, disorders of, gene therapy for, 763*t*, 764
  bacterial, as drug targets, 495
  denaturation of, by toxins, 725–726
  drug binding to, 34, 34*f*
  in drug design, 752–753, 752*t*
  as drugs, 757–762, 773–779
    advantages of, 757–758
    challenges of, 761–762
    classification of, 759–761, 759*t*, 773–779
    Group I (enzyme or growth factor activity), 759, 773–777
    Group II (special targeting activity), 760, 777–779
    Group III (vaccines), 760–761, 779
    Group IV (diagnostic), 761, 779
    production methods for, 758
    recombinant, 758
  structural, 13, 762–763, 763*t*
  structure of, 4–5, 4*f*
Protein C
  action of
    vitamin K in, 350–351, 351*f*
    warfarin effects on, 352–353
  activated recombinant, 353, 356, 775
  defects of, 345
  in hemostasis regulation, 341
Protein kinase A
  in peripheral sensitization, 235, 235*f*
  in platelet aggregation, 340*f*
Protein kinase C
  in drug-receptor interactions, 9, 11*f*
  in peripheral sensitization, 235, 235*f*
  in platelet activation, 339*f*
Protein S
  action of
    vitamin K in, 350–351, 351*f*
    warfarin effects on, 352–353
  defects of, 345
  in hemostasis regulation, 341
α1-Proteinase inhibitors, therapy with, 773
Proteinuria, edema in, 309
Prothrombin, in hemostasis, 339, 341*f*
Prothrombin time, in warfarin therapy, 353
Protirelin, 414
Proton pump inhibitors, for peptic ulcer disease, 688–690, 689*f*, 690*b*, 693
Protonation, 29, 29*f*
  of local anesthetics, 124–125, 126*f*
Proto-oncogenes, 728
Protozoa
  case study of, 593
  drugs for, 595–596, 601
  physiology of, 593–595, 594*f*, 595*f*
  types of, 587, 593–597
Protriptyline, 191
Proviruses, integrated, 765
Proximal tubule, in volume regulation, 304*f*, 305, 305*f*
PRPP (phosphoribosylpyrophosphate), in purine synthesis, 710, 710*f*
Pruritus, from niacin, 371
Pseudoaddiction, 256
Pseudoephedrine, 113, 118
Pseudohypoparathyroidism, 480*t*, 481
Pseudomembranous colitis, 542
*Pseudomonas aeruginosa*, beta-lactams for, 575

Pseudopodium, of *Entamoeba histolytica*, 594*f*
Psychedelic agents, abuse of, 248*t*
Psychologic dependence
  definition of, 250
  mechanisms of, 251–253
Psychosis, in schizophrenia, 170, 170*b*
Psychostimulants, abuse of, 248*t*
PTH. *See* Parathyroid hormone (PTH)
$P_{tissue}$ (tissue partial pressure), of anesthetics, 212–213, 213*t*
Pulmonary delivery, 782–783. *See also* Inhalation
Pulmonary embolism
  heparin for, 350
  from oral contraceptives, 453
Pulmonary fibrosis
  from bleomycin, 528
  from toxins, 726
Pulmonary vasculature, volume sensing in, 300–301
Pump, insulin, 785–786
Pump-based hypertension, 378
Pure Food and Drug Act of 1906, 792–793
  Amendment to (1912), 793*t*
Purified protein derivative, 779
Purified protein derivative test, for tuberculosis, 761
Purinergic neurotransmitters, 83
Purines
  metabolism of, 710–711, 710*f*
    in gout, 711–712, 711*t*, 712*f*
    inhibitors of, 513–514, 514*f*, 517
  in nucleotides, 506, 506*f*, 507, 509*f*
  structures of, 507*t*, 521*f*
  synthesis of, 507, 508*f*
Purkinje system, function of, 270–271
Putamen, 75–76.*76*f
  dopamine receptors in, 164, 165*f*
$P_{VRG}$ (partial pressure of vessel rich group), of anesthetics, 210, 211*f*, 213*t*, 214, 214*f*, 219
P2X ion channels, in pain physiology, 232
P2Y receptors
  in pain physiology, 232
  in platelet aggregation, 338, 340*f*
    inhibitors of, 347*f*, 348
Pyrantel pamoate, 599, 602
Pyrazinamide, 571*f*, 575, 578, 606
Pyrexia, prostaglandins in, 631*t*
Pyridostigmine, 100, 104
Pyridoxal phosphate, in catecholamine synthesis, 162, 162*f*
Pyrimethamine, 510*f*, 511, 511*f*, 511*t*, 512, 517
  in combination therapy, 512–513
  structure of, 510*f*
  sulfadoxine with, 607
  sulfonamides with, 607
Pyrimidines
  in nucleotides, 506–508, 506*f*, 510*f*
  structures of, 507*t*, 521*f*
  synthesis of, 507, 508*f*, 510*f*
Pyrrole acetic acid derivatives, 240
Pyruvate:ferredoxin oxidoreductase (PFOR), in *Entamoeba histolytica* metabolism, 594–595, 595*f*

**Q**

QRS complex, 271*b*, 272*f*
  in bundle of Kent conduction, 274, 274*f*
QT interval, 271*b*, 272*f*
  long, lidocaine for, 278
Quality control, in manufacture, 800
Quantal dose-response relationships, 19–20, 20*f*
Quaternary structure, of proteins, 4*f*, 5
Quazepam, 154*t*, 158
Quetapine, 174–175, 178
Quinapril, 315
*Quinghao* (artemisinin), 592, 592*f*, 600
Quinidine, 276–277, 283, 591–592, 600
Quinidine-like side effects, of tricyclic antidepressants, 187
Quinine, 591–592, 600
Quinoglide, 408, 413
Quinolines, 590–592, 591*f*, 592*f*, 600
Quinolones, 529–530, 534
Quinone, as primaquine metabolite, 593
Quinupristin, 542, 544

**R**

Rabeprazole, 689, 693
Racial factors, in hypertension management, 383
Radioactive iodide, for hyperthyroidism, 420–421
Radiosensitizers, hydroxyurea as, 515
Raloxifene, 450–451, 450*t*, 455
  for osteoporosis, 484, 484*f*, 488
  as partial agonist, 24
  structure of, 484*f*
Ramipril, 310, 315
Randomization of subjects, in clinical trials, 795
Ranitidine, 652, 653*f*, 654, 688, 688*f*, 693
RANK ligand, in bone remodeling, 476, 476*f*
Raphé nucleus, 77
  neurons of, 79, 79*t*, 80*f*
  neurotransmitter release from, 180
Rapid-detox protocol, 260
Rapidly turning over pool, of histamine, 648
Rasburicase, 714, 715
Rate of diffusion, of inhaled anesthetics, 209–210
Rational drug design, 7, 751–752
Ravuconazole, 583
Reabsorption
  of calcium, thiazides for, 313
  of sodium ion
    modulators of, 311–316
    pathologic, edema in, 307–309, 308*f*, 309*f*
    in volume regulation, 304–307, 304*f*–307*f*
Reactive species, in toxins, 722
Recall, of drugs, 800, 800*t*
Receptor(s)
  acetylcholine. *See* Acetylcholine (ACh), receptors for
  adrenergic. *See* Adrenergic receptors (adrenoceptors)
  AMPA. *See* AMPA receptor(s)
  binding, evaluation of, in drug development, 753
  bradykinin, 232
  cannabinoid, 233
  catecholamine. *See* Catecholamine(s), receptors for
  cholinergic
    muscarinic, 90–92, 91*f*, 92*t*, 173
    nicotinic, 90–93, 91*f*, 92*t*, 93*f*, 94*f*, 101, 102*t*, 105
  cognate, loss of function in, 762–763, 763*t*
  dopamine, 164, 165*f*
  drug, 3–16
    amino acids as, 80, 82*t*
    case study of, 3–4
    conformation and chemistry of, 4–7, 4*f*, 6*f*
      binding effects on, 5–6
      bond strength, 5, 5*t*
      membrane effects on, 6–7
    definition of, 3
    drug selectivity and, 14–15
    for drugs of abuse, 248*t*
    interactions of, 20–25, 21*f*–25*f*
      agonists, 7, 20–21
      antagonists. *See* Antagonists
      cellular regulation of, 13–14, 15*f*, 15*t*
      inverse agonists, 20, 24
      irreversible agonists, 21, 21*f*
      vs. nonreceptor-mediated mechanisms, 15–16
      partial agonists, 7, 20, 23–24, 23*f*
      receptor agonists, 21, 21*f*
      signal processing and, 13, 14*f*
      spare receptors, 19, 24–25, 24*f*, 25*f*
    receptor agonist, 21, 21*f*
    reversible, 21, 21*f*
    spare, 19, 24–25, 24*f*, 25*f*
    types of, 7–13, 7*f*, 8*t*
      with enzymatic cytosolic domains, 7*f*, 9–12, 12*f*
      G protein-coupled, 7*f*, 8–9, 10*f*, 11*f*, 11*t*, 68
      intracellular, 7*f*, 12–13, 13*f*
      ion channels, 7–8, 7*f*, 8*t*, 9*f*
  estrogen. *See* Estrogen(s), receptor for
  GABA. *See* GABA receptor(s)
  glutamate. *See* Glutamate receptor
  histamine, 649, 649*t*
    antagonists of, 651–654, 651*t*, 652*f*, 653*f*
  insulin, 460–462, 462*f*
  kainate, 141–142, 142*f*, 143*t*
  metabotropic. *See* Metabotropic receptors
  natriuretic peptide, 303, 303*f*
  NMDA. *See* NMDA receptor(s)
  norepinephrine, 183, 183*t*
  opioid. *See* Opioid(s), receptors for
  postsynaptic, 68
  progesterone, 449*f*, 452, 456
  serotonin, 182–183, 183*t*
  soluble N-ethylmaleimide-sensitive factor attachment protein (SNAREs), 67, 68*f*
  toxicity to, 724–725, 725*f*, 726*f*
Receptor agonist, definition of, 21, 21*f*
Receptor guanylyl cyclase, 12, 12*f*
Receptor serine/threonine kinases, 12, 12*f*
Receptor tyrosine kinases, 10, 12*f*
Receptor tyrosine phosphatases, 11, 12*f*
Recombinant drugs, 758, 773–777
  activated protein C, 353, 356, 775
  epidermal thymocyte activating factor (ETAF), 775
  factor VIIa, 775
  follicle-stimulating hormone, 759, 774
  glucagon, 775
  growth hormone-releasing hormone, 779
  human chorionic gonadotropin, 774
  human erythropoietin (rhEPO), 661–662, 665
  human G-CSF (filgrastim), 662–663, 665
  human GM-CSF (sargramostim), 662–663, 665
  human growth hormone, 406
  human thrombopoietin (rhTPO), 663, 666
  insulin, 773
  interferon-α, 774
  interferon-α-2a, 774
  interferon-α-2b, 774, 784
  interferon-β-1a, 774
  interferon-γ, 775
  interleukin-2, 775
  interleukin-11, 661*f*, 663, 666, 774
  parathyroid hormone, 775
  platelet-derived growth factor, 775
  proteins, 758
  tissue plasminogen activator, 353–354, 356, 390, 759
Recovery, from anesthesia, 218–219, 219*f*

Rectal formulations, 756t
Red blood cells. *See* Erythrocytes
Red neck (man) syndrome, from vancomycin, 572
Redistribution, of general anesthetics, 219
Redness, as classic sign of inflammation, 617
Reduced thiol-containing compounds, for nitrate tolerance, 326
Reducing agents, macromolecular damage from, 722
5α-Reductase
  in dihydrotestosterone synthesis, 442, 444f
  inhibitors of, 449, 449f
Reductions, in drug metabolism, 47t
Re-entry, in cardiac conduction, 273, 274f
Reflex tachycardia, in hypotension, 323
Refractoriness, of receptors, 14, 15t
Refractory ion channels, 8
Refractory period
  of ion channels, 14
  of myocytes, 269–270
    amiodarone effects on, 280
    calcium channel blocker effects on, 281
    effective, 276b
    sodium ion channel effects on, 275
  in neuron firing, 194
Refractory state, of membrane, in action potential, 66
Regional anesthesia, 132
Regular insulin, 466, 466t, 470
Regulation
  drug, 798–801
    costs, 800–801
    dietary supplements, 801
    indications for, 799–800
    labeling, 798, 799f
    marketing, 798–799
    naming, 798
    nonprescription medicines, 801
    patents, 801
    phase IV studies, 799, 800t
    production guidelines, 800
    withdrawals and recalls, 800, 800t
  volume. *See* Volume regulation
Regulatory toxicology, 743
Rejection, in transplantation, 668–669, 668t
Relapse, in addiction, 253
Relaxation, smooth muscle, in vascular tone regulation, 318–320, 319f, 320f
Release, in viral life cycle, 546, 547f, 548f
Release factors, in transcription, 538
Release reaction, in platelets, 336, 338
Relievers, for asthma, 699
Remifentanil, 239, 244
  as anesthetic adjunct, 228
  for pain, 234
Remodeling
  bone, 475–476, 476f
  myocardial, in congestive heart failure, 393–394, 394f
Renal. *See* Kidney
Renal toxicity. *See* Nephrotoxicity
Renin-angiotensin–aldosterone system
  in aldosterone regulation, 436
  in congestive heart failure, 394–395, 395f, 396f
  inhibitors of, 309–311, 310f, 315, 330
  in volume regulation, 301–303, 302f
Renovascular disease, hypertension in, 378
Repaglinide, 470
Replication-incompetent viral vectors, 767, 768f
Repolarization, in cardiac cells, 270, 270t, 271f
  inhibitors of, 279–281, 280f, 281t
  sodium ion channel blocker effects on, 275, 276f, 277f
  ventricular, T wave representing, 271b, 272f
Reproductive hormones, 441–456. *See also specific hormones*
  actions of, 442, 444, 444f
  in calcium homeostasis, 477t, 479
  for cancer, 610t
  disorders of
    case study of, 441–442
    pathophysiology of, 446–448, 447t
  drugs targeting, 449–456, 449f
    contraceptives, 452–453, 456
    inhibitors, 449–452, 450t, 451f, 455–456
    replacement, 453–454
  in hypothalamic-pituitary-reproductive axis, 408–410, 409f, 444–445, 444f, 445f, 447–448
  in menstrual cycle, 445–446, 446f, 447b
  physiology of, 442–446, 443f–446f, 447b
  structures of, 443f
  synthesis of, 442, 443f
Research, preclinical, 794
Reserpine, 118, 750t
  for hypertension, 381
  mechanism of action of, 109f, 112–113
  for mood disorders, 184
  in neurotransmitter regulation, 182
Resistance
  drug, 500–503, 502t
    acquired (extrinsic), 573
    acyclovir, 552
    aminoglycosides, 539
    antimalarial, 589
    antimony compounds, 596
    antimycobacterial, 575
    antineoplastic, 500–503, 502t, 609, 610t, 611, 611t
    beta-lactams, 573
    causes of, 501–503, 502t
    chloramphenicol, 541
    chloroquine, 591, 591f
    chromosomal (intrinsic), 573
    efflux pumps in, 540
    genetic factors in, 501–503, 502t
    macrolides, 541
    malaria parasites, 591, 591f
    methicillin, 574
    metronidazole, 595
    sulfonamides, 511–512
    tetracycline, 540
    vancomycin, 572
  in Ohm's law, 60, 60f
Resolution stage
  of hemostasis, 337f
  of inflammation, 625
Respiratory tract, toxin absorption in, 720–721
Response, to drug, vs. dose, 19–20, 19f, 20f
Rest angina, 326
Resting membrane potential, 61–62, 61f, 62t, 63f, 268, 269f
Restitutive properties, of gastric mucosa, 685
Reteplase, 775
Reticular activating system, 77, 96
Reticulocytes, 659
Retinoic acid, teratogenicity of, 728
Retinoic acid X receptor, 417, 418f
Retrosynthetic analysis, 754, 755f
Retroviruses. *See also* Human immunodeficiency virus and infection
  as gene therapy vectors, 765–768, 766t, 767f, 768f
  structure of, 554
Reversal potential, 67
Reverse cholesterol transport, 363
Reverse transcriptase, 554, 765, 767f
  in HIV replication, 546, 548f
  inhibitors of, 555–556, 557b, 557f, 564
Reverse triiodothyronine (rT3), 416, 416f
Reverse use-dependency, by potassium ion channel blockers, 280
Reversible agonist, definition of, 21, 21f
Reversible inhibitors of monoamine oxidase A (RIMAs), 188, 191
Reward pathway, in drug dependence, 250–253, 255, 256f, 257, 257f
Reye's syndrome, 639
rhEPO (recombinant human erythropoietin), 661–662, 665
Rheumatic fever, 670t
Rheumatoid arthritis, 670t
  case study of, 627–628
  inflammation in, 637
  leflunomide for, 673
  tumor necrosis factor α inhibitors for, 675–676, 675f
Rhinitis, allergic, histamine in, 650
Rhodanese, for cyanide poisoning, 739, 739f
rhTPO (recombinant human thrombopoietin), 663, 666
Ribavirin, 560, 564
Ribonucleosides, structures of, 507t
Ribonucleotide reductase, 508
  inhibitors of, 514–515, 517
Ribonucleotides
  reduction of, 508
  structures of, 507t
  synthesis of, 507–508, 509f, 510f
Ribosome
  70S, 536–537, 537f
  30S subunit of, inhibitors of, 539–541, 539t, 540f, 543
  50S subunit of, 537–538, 537f
    5S ribosomal RNA, 537
    23S ribosomal RNA, 537
    inhibitors of, 539t, 541–542, 544
  in transcription, 536–538, 536f, 537f
Rickets, 479–480, 480t, 486
Ridogrel, 642, 646
Rifabutin, 538
Rifampin, 538, 544, 606
Rifamycin B, 538
Riluzole, 147, 157
Rimantadine, 547f, 548–549, 550f, 562
RIMAs (reversible inhibitors of monoamine oxidase A), 188, 191
Risedronate, for bone disorders, 484–485, 488
Rising phase, of action potential, 65
Risperidone, 174–175, 178
Ritonavir, 547f, 556–557, 558f, 559b, 564, 748, 748f
Rituximab, 613, 777
Rivastigmine, 100, 104
River blindness (*Onchocerca volvulus*)
  drugs for, 598–599, 602
  physiology of, 597–598, 597f
Rizatriptan, 241, 245
RNA
  antisense, in gene therapy, 770–771, 770f
  in gene therapy. *See* Gene therapy
  inhibitory strategies of, in gene therapy, 770–771, 770f
  ribosomal. *See* Ribosome
  synthesis of, 506f, 508–509, 511f
  transfer, in translation, 537–538, 537f
  in viral life cycle, 546, 547f, 548f
RNA polymerase, in gene transcription, 536, 536f
Rocuronium, 101, 105
Rofecoxib, 236, 240, 244, 641, 641f, 644
ROMK (apical potassium ion channel), in volume regulation, 306, 306f
Ropinirole, 169, 176

Ropivacaine, 132, 134, 135
Rosiglitazone, for diabetes mellitus, 460f, 467–468, 471
Rosuvastatin, 369, 373
Roundworms, drugs for, 598–599, 602
rRNA. *See* Ribosomal RNA
RU-486 (mifepristone), 435, 439, 449f, 452, 456
*Rubor,* as classic sign of inflammation, 617
Ryanodine receptor, in cardiac contractility, 288

**S**

Safety
  in gene therapy, 764–765
  proving in preclinical stage, 794
Salicylates, 240, 638–639, 639f, 644, 730
Saline diuresis, for hypercalcemia, 487
Salmeterol, 115, 119, 700, 706
Salmon calcitonin, 485, 488
Salvage pathway
  for purine synthesis, 710, 710f
  for viral genome replication, 546
Saquinavir, 547f, 556, 564
Sarcolemma, of myocytes, 286–288, 287f, 289t, 291t, 293t
Sarcomere, of myocytes, 287, 287f, 289t
Sarcoplasmic reticulum (SR), of myocytes, 286, 287f, 289t, 291t, 293t
Sargramostim, 662–663, 665
Sarin, 724, 725f, 736t
Saturation kinetics, 38–39, 42f, 43
Saxitoxins, ingestion of, 729
Scale-up, in drug development, 755
Scavenger receptor, in lipoprotein removal, 361
Schizonts, in malarial parasite life cycle, 588, 588f
Schizophrenia, 170–175
  antipsychotic drugs for, 177–178
    atypical, 174–175, 178
    drug interactions of, 174
    mechanism of action of, 171–172, 172f, 173f
    pharmacokinetics of, 174
    side effects of, 172–173
  diagnostic criteria for, 170, 170b
  dopamine dysregulation in, 166
  dopamine receptors in, 164
  epidemiology of, 170
  pathophysiology of, 170–171, 170b
Scopolamine, 105
  action of, 83
  clinical applications of, 101
Screening, in drug design, high-throughput, 751
Secobarbital, 152t, 159
Second generation H1 antagonists, 652, 654
Second messenger(s), 9, 11f, 13, 14f
  in GABA$_B$ receptor activation, 147, 148f
Second messenger regulated ion channels, 7, 8t
Second pain, 122–123, 125f
Secondary (surrogate) endpoints, 796
Secondary generalized seizures
  definition of, 196t
  drugs for, 199–201
  pathophysiology of, 196–197, 197f, 198f
Secondary hemostasis, 336, 337f, 338–340, 341f, 342f
Secondary hyperparathyroidism, 481
Secondary hypertension, 377
Secondary osteoporosis, 482
Secondary structure, of proteins, 4, 4f
Secretins, 84
Secretory phase, of menstrual cycle, 445, 446f
Sedation, barbiturates for, 150, 151, 152t
Seizure(s)
  absence (petit mal)
    definition of, 196t
    drugs for, 201, 203–204
    pathophysiology of, 197, 199f
  case study of, 193–194
  classification of, 195, 196t
  complex partial (temporal lobe epilepsy), 196t
  drugs for, 198–204
    acting at T-type calcium ion channel, 201, 203
    enhancing GABA-mediated inhibition, 201–202, 204
    mediating sodium ion channel inhibition, 198–201, 200f, 203
    new, 204
  vs. epilepsy, 193
  generalized, 195, 196t
  myoclonic, 196t
  in normal brain, physiology of, 194–195, 194f, 195f
  partial
    definition of, 195, 196t
    drugs for, 198–201, 203–204
    pathophysiology of, 195–196, 197f
    with secondary generalization, 196t
  pathophysiology of, 195–197, 196t, 197f–199f
  primary generalized
    definition of, 196t
    pathophysiology of, 197, 197f, 199f
  secondary, pathophysiology of, 196–197, 197f
  secondary generalized
    drugs for, 199–201
    pathophysiology of, 196–197, 197f, 198f
  tonic–clonic (grand mal)
    definition of, 196t
    drugs for, 203–204
    pathophysiology of, 196–197, 198f
Selective estrogen receptor modulators (SERMs), 449f, 450–451, 450t, 451f, 455
  drug interactions with, 53
  for osteoporosis, 484, 484f, 488
  as partial agonist, 24
Selective serotonin reuptake inhibitors (SSRIs), 191
  action of, 82, 187f, 188
  examples of, 187
  pharmacokinetics of, 188
  side effects of, 188
Selective targets, of chemotherapeutic drugs, 494–495, 494t
Selective toxicity, 494, 494t, 724
Selectivity, drug, 14–15
Selegiline, 114, 118
  for depression, 188
  for Parkinson's disease, 169, 176
Self-tolerance, 669
  failure of, 669, 670t. *See also* Autoimmune diseases
Semilente insulin, 466t, 467, 470
Senile osteoporosis, 481, 482f
Sensitization
  central, 236, 236f
    NMDA receptors in, 145, 146f
  definition of, 249, 249f
  histamine in, 650
  peripheral, 235–236, 235f
Sensitizing agents, in pain, 231
Sensors, volume, 300–301
Sensory nervous system
  anatomy of, 73f, 73, 75
  long tract neuronal organization of, 78–79
Sensory transduction, 230–232, 231f, 231t
Sequential blockade, 513
Serine/threonine kinases, receptor, 12, 12f
Sermorelin, 406, 413
SERMs. *See* Selective estrogen receptor modulators (SERMs)
Serotonergic neurons, 80f
Serotonin, 80
  action of, 183
  degradation of, 182, 182f
  depressed neurotransmission of in depression, 184–185, 186f
  elevated, from MDMA, 259
  monoamine oxidase inhibitor effects on, 188–189
  neurons containing, 180
  in nociception, 123f
  in raphé nucleus, 79
  receptors for, 182–183, 183t
    agonists of, 245
  regulation of, 181–182, 182f
  reuptake of
    selective serotonin reuptake inhibitor effects on, 187–188
    tricyclic antidepressant effects on, 186–187, 187f
  structure of, 83f, 181f
  synthesis of, 82, 84f, 180, 181f, 182, 182f
Serotonin autoreceptors, 185, 186f
Serotonin hypothesis, of antipsychotic action, 174
Serotonin syndrome, 114, 188
Serotonin transporter, 182, 182f, 257
  inhibitors of, 187f, 188
Sertoli cells, hormone action on, 445, 445f
Sertraline, 187, 191
Serum sickness, 724
Severe combined immunodeficiency, gene therapy for, 768, 768f
Severe persistent asthma, management of, 704, 704t
Sevoflurane, 227
  blood/gas partition coefficient of, 220
  perfusion-limited, 214
  pharmacology of, 220
  properties of, 208t
  structure of, 222f
  tissue/blood partition coefficients of, 213t
Sex hormone(s). *See* Reproductive hormones; *specific hormones, e.g.,* Estrogen(s); Testosterone
Sex hormone binding globulin (SHBG), testosterone binding to, 444f
SHBG (sex hormone binding globulin), testosterone binding to, 444f
Shock
  β-adrenergic receptor agonists for, 115
  dopamine for, 112
  heart failure in, 392t
  hemorrhagic, anesthesia induction in, 216
  inotropic agents for, 298
Short-term testing, preclinical, 794
"Shotgun approach," to drug discovery, 748, 751
Shunting, in GABAergic transmission, 148
SIADH (syndrome of inappropriate ADH), 410
Sialic acid, neuraminidase action on, 559, 559f
Sickle cell disease, 658
  hemoglobin F inducers for, 662, 665
  hydroxyurea for, 515
Siemens, as reciprocal Ohms, 60
Signal transduction molecules, 13
Sildenafil, 331
  action of, 323f
  development and approval of, 791–792
  for vascular tone modulation, 326
"Silent killer," hypertension as, 376

Silica dust, inhalation of
  autoimmune disease from, 724
  pulmonary fibrosis from, 726
  toxicity of, 737*t*
Silicon microchip drug delivery system, 786
Simple partial seizures, definition of, 196*t*
Simvastatin, 369, 373, 389
Single nucleotide polymorphisms (SNPs), 804, 804*f*
Single-blind trials, 795–796
Single-source divergent neuronal organization, 78*f*, 79–80, 79*t*, 80*f*
Sinoatrial node
  dysfunction of
    beta-blocker effects on, 278, 278*f*
    calcium channel blocker effects on, 281, 281*f*
    impulse formation defects in, 272–273, 273*f*
  function of, 268–270, 269*f*, 270*t*, 273–274
  pacemaker cells in, 268
Sinus tachycardia, 276*b*
Sirolimus (rapamycin), 674, 675*f*, 680, 786
Skeleton. *See* Bone(s)
Skin
  drug administration through (subcutaneous), 31, 31*t*
  drug administration to, 31*t*, 32, 783
  toxin absorption through, 720–721
Sleep
  acetylcholine effects on, 96
  slow-wave (stage 3), vs. absence seizure, 197, 199*f*
Sleeping sickness, drugs for, 596, 601
"Slow acetylators," 806–807, 807*f*
Slow excitatory postsynaptic potential, 94, 97*f*
Slow inhibitory postsynaptic potential, 94, 97*f*
Slow vs. fast acetylators, 52
Slowed retrograde conduction velocity, re-entry in, 273, 274*f*
Slowly turning over pool, of histamine, 648
Slow-release preparations, of nitroglycerin, 324
Slow-wave (stage 3) sleep, vs. absence seizure, 197, 199*f*
Smoking
  cessation of, for peptic ulcer disease, 691
  nicotine addiction in. *See* Nicotine
  reward system in, 257, 257*f*
  toxic effects of, 726, 729, 732*t*
Smooth muscle
  function of, in vascular tone. *See* Vascular tone
  histamine effects on, 648, 648*t*
  nonvascular, nitrate effects on, 326
SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors), 67, 68*f*
SNPs (single nucleotide polymorphisms), 804, 804*f*
Social treatment, for addiction, 259, 260, 261*b*
Sodium bicarbonate, 690, 693, 730
Sodium current, 63–64, 268–270, 269*f*, 270*t*
Sodium ion
  contribution to resting potential, 63*f*
  excretion of, for volume regulation, 304–307, 304*f*–307*f*
  Nernst equilibrium potential for, 62, 62*t*
  reabsorption of
    modulators of, 311–316
    pathologic, edema in, 307–309, 308*f*, 309*f*
  retention of
    edema in, 307–309, 309*f*
    hypertension in, 378
Sodium ion channel(s)
  in action potential, 63–66, 64*f*, 65*f*
  in afterdepolarization, 272–273, 273*f*
  in AMPA receptor activation, 142, 143*t*
  antiepileptic drug inhibition of, 198–201, 200*f*, 203
  blockers of, 275–278, 276*f*, 277*f*
  in cardiac function, 268–270, 269*f*, 270*t*
  conformational states of, 128, 129*f*
  in dopamine release, 164
  in kainate receptor activation, 142, 143*t*
  neuronal firing and, 194–195, 194*f*
  in neuropathic pain, 237, 237*f*
  neurotransmitter effects on, 140, 140*f*
  in nicotinic transmission, 92–93, 93*f*, 94*f*
  in pain transmission, 232
  in peripheral sensitization, 235, 235*f*
  pharmacology of, 66
  states of, 194–195, 194*f*
  voltage-gated, in pain transmission, 128, 129*f*, 129*t*, 130*f*
Sodium nitrite, for cyanide poisoning, 739, 739*f*
Sodium nitroprusside, 324, 324*f*, 325*f*, 331
Sodium polystyrene sulfonate, for toxic exposures, 740
Sodium stibogluconate, 596, 601
Sodium thiosulfate, for cyanide poisoning, 739, 739*f*
Sodium/bicarbonate cotransporter (NBC1), in volume regulation, 305, 305*f*
Sodium–calcium exchanger, 62
  in cardiac contractility, 287–288, 290*f*
  digoxin effects on, 293, 293*f*
Sodium-chloride cotransporter (NCC1), in volume regulation, 306, 306*f*
Sodium/hydrogen exchanger, in volume regulation, 305, 305*f*
Sodium-potassium ATPase, 62
  aldosterone effects on, 436
  in cardiac contractility, 287–288, 290*f*
  digoxin binding to, 293, 293*f*
  in dopamine transport, 163, 163*f*
  in volume regulation, 306, 306*f*, 30, 307*f*
Sodium-potassium-chloride cotransporter (NKCC2), in volume regulation, 306, 306*f*
Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), 67, 68*f*
Solvent activation, in polymer-based delivery systems, 784*f*, 785
Solvent/gas partition coefficient, of general anesthetics, 207, 208*t*, 209*b*, 210*f*
Soman, 724, 725*f*, 737*t*
Somatic nervous system, neurotransmitters in, 81*f*
Somatostatin, 84, 404, 406, 407*f*, 413, 758
  action of, 462
  cells secreting, 458
  in energy homeostasis, 458–459, 458*t*, 460*f*
  in gastric acid secretion regulation, 684
  in *Helicobacter pylori* infections, 686, 686*f*
  secretion of, 458
Somatotrophs, 405*t*
  adenoma of, 407
Sonophoresis, 783
Sotalol, for arrhythmias, 280
Spare receptors, 19, 24–25, 24*f*, 25*f*
Sparteine, metabolism of, polymorphism in, 807–808
Specific lymphocyte signaling inhibitors, for immunosuppression, 674, 674*f*, 675*f*, 680
Specificity, of drug-receptor interactions, 6–7
Spectinomycin, 537*f*, 539*t*, 540, 543
Spectrum of action, of beta-lactams, 573
Speech, disorganized, in schizophrenia, 170
Spinal anesthesia, 132
Spinal cord
  anatomy of, 77–78, 77*f*
    cross-section, 73*f*
    nerves originating in, 73, 75
    segmental, 75*f*
  in pain transmission pathway, 95–96, 232–233, 233*f*, 234*f*
Spinal nerves, 73
Spironolactone, 313, 316, 439
  action of, 307*f*
  as androgen receptor antagonist, 449*f*, 452, 455
  for congestive heart failure, 396, 396*f*, 397*t*
  for hypertension, 380
  side effects of, 436–437
Spontaneous pain, 234
Sporozoites, in malarial parasite life cycle, 588, 588*f*
SREBPs (sterol regulatory element binding proteins), 368, 369*f*
ST segment, in electrocardiogram, 271*b*, 272*f*
Stability profile, in drug development, 754
Stabilizers, for pills, 756
Stable angina, nitrates for, 388–389
Stable angina, pathophysiology of, 385, 385*f*
*Staphylococcus aureus*
  methicillin-resistant, 574
  toxins of, 729
Starch blockers (α-glucosidase inhibitors), for diabetes mellitus, 460*f*, 465–466, 470
Starling relationship, in cardiac contractility, 287
Stasis, of blood flow, thrombosis in, 343, 343*f*
State-dependent binding, of ion channels, 8
State-dependent ion channel block, 275
Static drugs
  antifungal, 583, 604
  antimicrobial, 496, 604–605, 604*f*, 604*t*, 605*f*
Statins (HMG CoA reductase inhibitors), 368–369, 369*f*, 373, 389
Statins, pharmacogenomics of, 805–806
Statistical review, of new drugs, 796
Stavudine (d4T), 551*f*, 563
Steady-state drug concentration, 41–42, 42*f*, 43*t*
Stealth liposomes, 786
Steel factor (stem cell factor, c-kit ligand), 657, 658
Stem cell(s)
  in immune response, 618, 619*f*
  lymphoid, 659
  myeloid, 659
  pluripotent hematopoietic, 656, 657*f*
Stem cell factor (steel factor, c-kit ligand), 657, 658
Stents, sirolimus-eluting, 674, 786
Stepped care, for hypertension, 382–383
Stereochemistry, 5
Steroid hormones, 12–13, 13*f*
Steroid 21-hydroxylase, deficiency of, congenital adrenal hyperplasia in, 437–438, 437*f*
Sterol(s), mammalian vs. eukaryotic, 580
14α-Sterol demethylase, in ergosterol synthesis, 580
  inhibitors of, 581–582, 581*f*, 585
Sterol regulatory element binding proteins (SREBPs), 368, 369*f*
Stibogluconate, 596, 601
Stimulants. *See* Amphetamines; Cocaine
Strand-passage model, of DNA supercoiling, 522*f*
Strand-rotation model, of DNA supercoiling, 522*f*
Stratum corneum, as toxin barrier, 721
Streptogramins, 537*f*, 539*t*, 542, 544
Streptokinase, 353, 356, 390, 748, 758, 776
Streptomycin, 539, 543, 606

Stress
  glucocorticoid release in, 428–429
  peptic ulcer disease in, 687
Striatum, 75–76, 80*f*
  dopamine receptors in, 164, 165*f*
Stroke, glutamate receptors in, 144–145, 145*f*, 146*f*
Stroke volume
  abnormal, in congestive heart failure, 393–394, 394*f*
  as blood pressure determinant, 377, 377*f*
  in cardiac contractility, 287
  determinants of, 392–393, 392*f*
Structural proteins, 13
  loss of function in, gene therapy for, 762–763, 763*t*
Structure-based drug design, 751–752
Subcutaneous drug administration, 31, 31*t*, 756*t*
Subendocardial myocardial infarction, 386
Subjects, in clinical trials, 795
Sublingual formulations, 324, 756*t*
Substance abuse. *See* Addiction; Dependence; Tolerance
Substance P, in pain transmission, 123*f*, 130, 233*f*
Substantia nigra
  anatomy of, 76
  dopaminergic neurons in, 166, 166*f*
  neuronal organization of, 79, 79*t*, 80*f*
Substantia nigra pars compacta, dopamine deficiency in, 166, 167*f*
Subthalamic nucleus, in movement control, 166, 167*f*
Succimer, 741
Succinic semialdehyde, in GABA synthesis, 141, 141*f*, 147
Succinylcholine, 105, 228
  action of, 100, 101
  with general anesthesia, 221
  metabolism of, genetic variations in, 52
Sucralfate, 690, 694
Sudden cardiac death, in myocardial infarction, 386
Sufentanil, 239, 244
Suicide substrate inhibition (dead-end complex), 570*f*, 573
Sulbactam, 572*f*, 573
Sulfa drugs, 509
Sulfadiazine, 510, 517, 510*f*
Sulfadoxine/pyrimethamine, 591*f*, 607
Sulfamethoxazole, 510, 517
  structure of, 510*f*
  trimethoprim with, 607
Sulfanilamide, 510*f*
Sulfate conjugation, in drug metabolism, 48*t*, 49, 51*f*
Sulfenamide, for peptic ulcer disease, 688–689, 689*f*, 690*b*
Sulfhydryl theory, of nitrate tolerance, 326
Sulfinpyrazone, for gout, 710*f*, 714, 715
Sulfonamides, 509–511, 509*f*–510*f*, 517
  in combination therapy, 607
  loop diuretics as, 312
  toxicity of, 737*t*
Sulfones, 511
Sulfonylureas, for diabetes mellitus, 460*f*, 461*f*, 467, 468, 469*t*, 470
Sulfur dioxide, toxicity of, 737*t*
Sulfuric acid
  macromolecular damage from, 722
  toxicity of, 737*t*
Sulindac, 640
Sumatriptan, 241, 245
Supercoiling, of DNA, 521, 522*f*
Superior cervical ganglion, 72
Superior mesenteric ganglion, 72, 74*f*
Superoxide dismutase, 595
Superoxide ion
  in heme metabolism, 589, 589*f*
  from nifurtimox, 596
Superoxide theory, of nitrate tolerance, 326
Supplements, dietary, regulation of, 801
SUR subunits, of potassium ion/ATP channel, 459–460, 461*f*, 467–469
Suramin, 596, 601
Surface heparin-like molecules, 337*f*
Surgical anesthesia stage, of general anesthesia, 206*f*
Surrogate (secondary) endpoints, 796
Surround inhibition, of neurons, 195, 195*f*
Swelling, as classic sign of inflammation, 617
Switzerland, drug approval in, 798
Symbols, for general anesthetic pharmacology, 225
Sympathetic nervous system
  anatomy of, 72–73, 73*f*, 74*f*
  in arrhythmias, beta-blocker effects on, 279
  in automaticity regulation, 272
  neurotransmitters in, 81*f*
  in nitrate tolerance, 325
  renal, in volume regulation, 304
  in vascular tone regulation, 322
Sympatholytics, 112, 113*f*, 115–117, 117*t*, 119, 380–381
Sympathomimetics, 113*f*
  for congestive heart failure, 295–296, 295*t*, 398
  indirect, 113–114, 113*f*
  mixed, 113–114
Synapsin, 67
Synaptic cleft, 67
Synaptic neuromodulators, in pain transmission, 232
Synaptic transmission. *See* Electrochemical transmission
Synaptic vesicles, regulation of, 67–68, 68*f*
Synaptobrevin, 67
Syndrome of inappropriate ADH, 410
Synercid, 542, 544
Synergism, 605–606, 605*f*
  aminoglycosides with beta-lactams, 539, 573–574, 607
  examples of, 606–607
Synthesis, chemical, in drug development, 754–755
Synthetic compounds, library of, for drug design, 749
Systemic arterial hypertension, heart failure in, 391
Systemic lupus erythematosus, 670*t*
Systemic vascular resistance (SVR), 318
  β-adrenergic receptor agonist effects on, 295
  as blood pressure determinant, 377, 377*f*
  calcium channel blocker effects on, 381
  increased, hypertension in, 378
Systolic blood pressure, significance of, 376, 377*t*
Systolic heart failure, 391–392
Systolic ventricular wall stress, in myocardial oxygen demand, 318, 319*f*

## T

T4. *See* 3,5,3′,5′-Tetraiodothyronine (thyroxine, T4)
$t_{1/2}$ (half-life), 39, 39*t*, 40*b*, 43*t*
T-20 (enfuvirtide), 547–548, 547*f*, 549*f*, 562
T cells, 622, 622*f*, 656*t*, 657*f*
  costimulation of, inhibitors of, 677–679, 678*f*, 681
  cytotoxic, 620*t*, 622, 622*f*
  in delayed-type hypersensitivity, 723*f*, 724
  function of, 659
  helper, 620*t*, 622, 622*f*, 697–698, 697*f*
  mycophenolate effects on, 672, 672*f*, 672*t*
  production of, 659–661
T wave, in electrocardiogram, 271*b*, 272*f*
Tabun, 724, 725*f*, 737*t*
TAC (tetracaine-adrenaline-cocaine), 131, 135
Tachyarrhythmias, in re-entry, 273, 274*f*
Tachycardia
  paroxysmal supraventricular, 276*b*
  reflex, in hypotension, 323
  sinus, 276*b*
  ventricular, 276*b*
Tachykinins, 84
  in pain transmission, 232
Tachyphylaxis, 14, 15*t*
  from stimulants, 258
Tacrine, 100, 104
Tacrolimus (FK506), 674, 674*f*, 680
Tadalefil, 331
Tagging, in combinatorial chemistry, 751
Talc
  pulmonary fibrosis from, 726
  toxicity of, 737*t*
Tamoxifen, 450, 450*t*, 455, 610*t*
Tamsulosin, 116, 119
Tangier disease, 365*t*, 366
Tardive dyskinesia, from antipsychotics, 173
Target(s)
  drug
    altered, 502
    antifungal, 498
    antimicrobial, 494–496, 494*t*, 496*f*
    antineoplastic, 494–495, 494*t*, 499, 500*f*, 501*f*, 502
    antiparasitic, 498
    antiretroviral, 497–498, 497*f*
    chemotherapeutic, 494–495, 494*t*
    design and, 748–752, 751*f*
    insulin, 459–462, 462*f*
    polymorphisms in, 805–806, 805*t*
    protein, 760, 777–779
    reproductive hormones, 449–456, 449*f*
    of tissues, 786
  of thyroid hormones, 417–418, 418*f*
Taxanes, 530*f*, 531, 534, 610*t*
Tay-Sachs disease, gene therapy for, 763, 763*t*
Tazobactam, 573
TBG (thyroid binding globulin), 417
3TC (lamivudine), 551*f*, 555, 563
TCAs. *See* Tricyclic antidepressants (TCAs)
TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), toxicity of, 724
$TD_{50}$ (median toxic dose), 20, 20*f*
  in therapeutic index, 25
Teicoplanin, 568*f*, 572, 577
Telmisartan, 315
Temazepam, 154*t*, 158
Temperature, nociceptor firing and, 122, 124*f*
Temporal lobe, 75, 76*f*
Temporal lobe epilepsy (complex partial seizures), 193–194, 196*t*
Tenecteplase, 776
Teniposide (VM-26), 529, 533
Tenofovir, 551*f*, 554, 563
Tensile strength, of bacterial cell wall, 566
Teratogenesis, 447*b*, 728
  of alcohol, 729
  in toxic exposures, 722
Terazosin, 116, 119, 329, 333, 381
Terbutaline, 115, 119, 699, 706
Terfenadine, 652, 654
Teriparatide, 775

Termination stage, of transcription, 536, 536*f*, 538
Tertiary structure, of proteins, 4, 4*f*
Testis
  cancer of
    case study of, 519–520
    combination therapy for, 612, 613*f*
  hormone action on, 445, 445*f*
Testosterone
  actions of, 442, 444*f*
  inhibitors of, 449, 449*f*, 455
  prostate hyperplasia and cancer and, 448
  regulation of, 444*f*, 445, 445*f*
  release of, 409*f*
  replacement of, 454
  synthesis of, 427*f*, 442, 443*f*, 445, 445*f*
  in transdermal patch, 454
Testosterone cypionate, for replacement, 454
Testosterone enanthate
  for male contraception, 453
  for replacement, 454
Tetanic fade, 94, 97*f*
Tetanus toxin, 149*t*
Tetracaine, 133, 135
  in TAC (tetracaine-adrenaline-cocaine), 131, 135
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), toxicity of, 724
Tetrachloroethylene, toxicity of, 721, 737*t*
Tetracyclines, 537*f*, 539*t*, 540–541, 543, 691
Tetrahydrazoline, 115, 119
Tetrahydrobiopterin, in catecholamine synthesis, 162, 162*f*
Tetrahydrofolate, in purine synthesis, 507, 509*f*
3,5,3′,5′-Tetraiodothyronine (thyroxine, T4)
  abnormal levels of. *See* Hyperthyroidism; Hypothyroidism
  feedback regulation of, 418, 419*f*
  metabolism of, 417, 417*f*
    inhibitors of, 420*f*, 421, 423
  replacement of, 419–420, 423
  secretion of, 416–417, 417*f*
  structure of, 416, 416*f*
  synthesis of, 416, 416*f*, 417*f*
  target tissue effects of, 417–418, 418*f*
Thalamocortical projections, seizure spread through, 196, 197*f*
Thalamus
  anatomy of, 76
  in pain transmission pathway, 123, 126*f*
  seizure spread through, 199*f*
Thalassemia, 658
Thalidomide, 797
  enantiomers of, 5
Theophylline
  for asthma, 699*f*, 700, 706
  inotropic effects of, 296
Therapeutic index (TI), 25, 494
  of general anesthetics, 207, 208*f*
Therapeutic window, 25
  vs. steady-state drug concentration, 42*f*
Thermodynamics, of distribution, 34–35, 35*f*–37*f*
Thermosensitive neurons, 230–231, 231*f*
Thiabendazole, 599, 602
Thiazide diuretics, 306*f*, 313, 316, 379–380
  for congestive heart failure, 395–396
Thiazolidinediones, for diabetes mellitus, 460*f*, 467–468, 471
Thick ascending limb of the loop of Henle, in volume regulation, 304*f*, 305–306, 306*f*, 312–313, 316
Thioamines, 420*f*, 421, 423
Thiocyanate, 420, 420*f*, 423
Thioguanine, 506*f*, 509*f*, 514*f*, 515, 518
Thiol-containing compounds, reduced, for nitrate tolerance, 326
Thiomethoxyflurane, oil/gas partition coefficient of, 210*f*
Thiopental, 159, 227
  clinical applications of, 151, 152*t*
  intravenous, pharmacology of, 220
  structure of, 222*f*
Thioridazine, 177
Thiotepa, 524, 526, 533
Thiothixene, 177
Thioxanthines, 171, 173*f*
Thoracolumbar (sympathetic) nervous system, anatomy of, 72–73, 73*f*, 74*f*
Threshold potential, 65
Threshold value, of action potential, 63, 64*f*
Thrombin
  in hemostasis, 336, 337*f*, 339, 340*f*, 341*f*
  inhibitors of, 353, 356
Thrombocytes. *See* Platelet(s)
Thrombocytopenia
  heparin-induced, 345, 350
  platelet-inducing agents for, 663, 666
Thrombocytopenic purpura, autoimmune, 670*t*
Thrombolytic agents, 353–354, 356
  contraindicated in unstable angina, 389
  for myocardial infarction, 390
Thrombomodulin, in hemostasis, 337*f*, 341
Thrombopoiesis, 661, 661*f*
  inducers of, 663, 666
Thrombopoietin, 658, 661
Thrombosis
  case study of, 335–336
  deep vein, from oral contraceptives, 453
  definition of, 335
  drugs for, 345–356
    antiplatelet agents, 326, 345, 346*f*, 347*f*, 348, 355, 638–640, 639*f*
    protein-based, 775
    thrombolytic agents, 353–354, 356, 390
  in factor V Leiden mutation, 808
  pathogenesis of, 343–345, 343*f*, 344*t*
  warfarin-induced, 352–353
Thromboxane(s)
  actions of, 631, 632*f*
  antagonists of, 642, 646
  degradation of, 633
  formation of, in platelet activation, 336, 337*f*, 338*f*
  inhibitors of, 347*f*
  in platelet aggregation, 338, 339*f*
  synthesis of, 345, 346*f*, 628*f*, 630*f*, 631
Thrombus, definition of, 343
Thymidine kinase, activity of, 552
Thymidylate, synthesis of, 508
Thymidylate synthase, 508
  inhibitors of, 513, 513*f*, 517, 583, 584*f*, 585
Thymine
  in nucleotides, 506, 520, 520*f*
  structure of, 507*t*, 520, 520*f*, 521*f*
Thyroglobulin, 416–417, 417*f*
Thyroid binding globulin (TBG), 417
Thyroid gland, 415–423
  hormones of, 416–418, 416*f*–419*f*. *See also specific hormones*
    abnormal levels of. *See* Hyperthyroidism; Hypothyroidism
    in calcium homeostasis, 477*t*, 479
  in hypothalamic-pituitary-thyroid axis, 408, 418, 419*f*
  physiology of, 416–418, 416*f*–419*f*
Thyroid peroxidase, 416, 417*f*
Thyroid hormone receptors, 417, 418*f*
Thyroiditis, Hashimoto's, 418–419, 419*f*
Thyroid-stimulating hormone (TSH), 404, 408, 414, 418, 419*f*
  recombinant, 779
Thyroid-stimulating immunoglobulin (TsIg), 418
Thyrotrophs, 405*t*
Thyrotropin-releasing hormone (TRH), 84, 405*t*, 407, 408, 418, 419*f*
Thyroxine. *See* 3,5,3′,5′-Tetraiodothyronine (thyroxine, T4)
TI (therapeutic index), 25, 494
  of general anesthetics, 207, 208*f*
Tiagabine, for seizures, 204
Ticarcillin, 573, 575, 577
Ticlopidine, antiplatelet activity of, 347*f*, 348, 355
Time-dependent killing, of drugs, 604, 604*f*, 604*t*, 605*f*
Timolol, 116, 117*t*, 119, 387*t*
Tirofiban
  antiplatelet activity of, 348, 355
  for unstable angina, 389
Tissue damage
  chemical mediators in, 623*t*
  nociception in, 122, 123*f*
Tissue factor, in hemostasis, 337*f*, 338*f*, 339, 341*f*
Tissue factor pathway inhibitor, in hemostasis regulation, 342
Tissue partial pressure, of anesthetics, 212–213, 213*t*
Tissue schizonts, in malarial parasite life cycle, 588, 588*f*
Tissue/blood partition coefficients, of general anesthetics, 212–213, 213*t*, 214*f*
Tissue-type plasminogen activator
  in fibrinolysis, 337*f*
  in hemostasis regulation, 342, 343*f*
  for myocardial infarction, 390
  recombinant, 353–354, 356, 390, 759
Tizanidine, 157
Tobacco, nicotine addiction in. *See* Nicotine
Tobramycin, 539, 543
Tocainide, 278, 283
Tolazamide, 470
Tolbutamide, 470
Tolcapone, 169, 176
Tolerance
  to drugs
    barbiturates, 153
    benzodiazepines, 155
    definition of, 249, 249*f*
    inverse, 249, 249*f*
    list of, 248*t*
    mechanisms of, 250–251, 251*f*, 252*f*
    morphine, 253*f*
    nitrates, 325–326, 388–389
  of immune system, 621
    central, 669
  peripheral, of immune system, 669
  self-
    breakdown of, 669, 670*t*
    of immune system, 669. *See also* Autoimmune diseases
Tone
  of neuron activity, 180
  vascular. *See* Vascular tone
Tonic firing, in GABAergic transmission, 148
Tonic inhibition, of sodium ion channels, 128, 130*f*
Tonic phase, of seizure, 197
Tonic–clonic (grand mal) seizures
  definition of, 196–197, 196*t*
  drugs for, 203–204
  pathophysiology of, 196–197, 198*f*
Tophi, 711
Topical anesthetics, 131

- Topical glucocorticoids, 434
- Topiramate, 204
- Topoisomerases
  - in DNA replication and coiling, 521, 522*f*
  - inhibitors of, 522*f*, 528–530, 533–534
  - in transcription, 536
- Topotecan, 529, 533
- Toremifene, 450, 450*t*, 455
- *Torsades de pointes,* 272–273, 276*b*, 277
- Torsemide, 312, 316
- Tositumomab, 613
- Toxic exposures. *See also* Toxicity
  - case study of, 720
  - cigarette smoke, 729. *See also* Smoking
  - ethanol, 729. *See also* Alcohol
  - food poisoning, 729
  - lead, 729–730
  - pathophysiology of, 720–722
  - prevalent, 729–730
  - treatment of, 729, 731*t*–738*t*
    - antidotes for, 731*t*–738*t*
    - pharmacologic, 741–743
    - poison inactivation in, 740–741, 741*f*
    - toxicokinetic-based, 730, 738–740, 739*f*, 740*f*
- Toxic metabolites, 45, 51–52
- Toxication, 721–722
  - definition, 720
  - inhibition of, 738
- Toxicity. *See also* Median toxic dose ($TD_{50}$); Toxic exposures
  - acute, 720
  - of barbiturates, 153
  - of benzodiazepines, 155
  - birth defects from (teratogenesis), 447*b*, 728
  - carcinogenesis in. *See* Carcinogenesis
  - cellular death and repair in, 725–726
  - chronic, 720
  - of local anesthetics, 132–133
  - mechanisms of, 722–728, 723*f*, 725*f*, 726*f*, 731*t*–738*t*
  - organ-directed, 726–727
  - saturation kinetics and, 42*f*, 43
  - selective, 494, 494*t*, 724
- Toxicodynamics, definition of, 720
- Toxicokinetics
  - absorption in, 720–721, 730, 738
  - definition of, 720
  - distribution in, 721
  - elimination in, 721
    - enhancement of, 739–740
  - exposure in, 720
  - treatments based on, 730, 738–740, 739*f*, 740*f*
- Toxicology
  - definition of, 719, 720
  - evaluation of, in drug design, 754
  - types of, 719, 743
- Toxicology review, of new drugs, 795
- Toxidrome, 730
- Toxin conjugates, for cancer, 610*t*
- tPA. *See* Tissue-type plasminogen activator
- Trade name, 798
- Trandolapril, 315
- Transcellular biosynthetic routes, for eicosanoid synthesis, 636, 636*f*
- Transcortin (corticosteroid-binding globulin), 427
- Transcription
  - in prokaryotes, 536–537, 536*f*, 537*f*
    - inhibitors of, 538, 544
  - in viral life cycle, 546, 548*f*
- Transcription factors, 12–13, 13*f*
- Transdermal drug administration, 31*t*, 32, 783
  - formulations for, 756*t*
  - nitroglycerin, 324
  - testosterone, 454
- Transduction
  - in drug resistance, 501
  - sensory, 230–232, 231*f*, 231*t*
- Transfer RNA, in translation, 537–538, 537*f*
- Transformation
  - in carcinogenesis, 498–499
  - in drug resistance, 501
- Transfusion, exchange, for toxin elimination, 740
- Transglycosidase, inhibitors of, 572
- Translation
  - in prokaryotes, 537–538, 537*f*
    - inhibitors of, 538–544, 539*t*, 540*f*
  - in viral life cycle, 546, 548*f*
- Translocation, 538
- Transmigration, of leukocytes, in inflammation, 624, 625*f*
- Transmission
  - chemical, 69
  - electrochemical, 66–69, 66*f*, 68*f*
- Transmission mode, of neurons, in absence seizure, 197
- Transmural myocardial infarction, 386
- Transpeptidases (penicillin-binding proteins, PBPs), in murein crosslinking, 567, 568*f*, 569, 570*f*
  - inhibitors of, 566, 568*f*
- Transplantation
  - graft-versus-host disease in, 669
  - immunosuppressive drugs for, 667–668, 679*t*. *See also* Immunosuppressive drugs
  - rejection in, 668–669, 668*t*
- Transport, active, 28
- Transthyretin, 417
- Transudative edema, pathophysiology of, 307–309, 308*f*, 309*f*
- Tranylcypromine, 114, 118, 191
- Trapping, pH, 29, 29*f*
- Trastuzumab, 752*t*, 760, 779
- Trauma, brain, glutamate receptors in, 144–145, 145*f*, 146*f*
- Travoprost, 645
- Trazodone, 189, 191
- Treatment INDs, 794, 797
- Tretinoin, for cancer, 610*t*
- TRα and TRβ thyroid hormone receptors, 417, 418*f*
- TRH (thyrotropin-releasing hormone), 405*t*, 407, 408, 418, 419*f*
- Triamcinolone, 439
  - for asthma, 701, 706
  - inhaled, 433
  - structure of, 434*f*
- Triamterene, 313–314, 316
  - action of, 307*f*
  - for hypertension, 380
- Triazolam, 154, 154*t*, 158
- Trichloroethylene, poisoning from, 734
- Tricyclic antidepressants (TCAs), 191
  - action of, 82, 114, 186, 187*f*
  - discovery of, 184
  - for pain, 245
  - pharmacokinetics of, 187
  - side effects of, 186–187
  - structures of, 186
  - toxicity of, 737*t*
- Trientine, 741
- Trifluoperazine, 177
- Trifluridine, 562
- Triglycerides
  - degradation of, 359, 360, 361*f*
  - elevated. *See* Hypertriglyceridemia
  - in lipoproteins, 358*t*
- Trihexyphenidyl, 169–170, 176
- 3,3′,5-Triiodothyronine (reverse T3), 416, 416*f*
- 3,5,3′-Triiodothyronine (T3)
  - abnormal levels of. *See* Hyperthyroidism; Hypothyroidism
  - metabolism of, 417, 417*f*
    - inhibitors of, 420*f*, 421, 423
  - structure of, 416, 416*f*
  - synthesis of, 416, 416*f*, 417*f*
  - target tissue effects of, 417–418, 418*f*
- Trilostane, 427*f*, 435, 435*t*, 439
- Trimethaphan, 105
  - clinical applications of, 103
  - for hypertension, 381
- Trimethoprim, 510*f*, 511, 511*t*, 512, 517
  - in combination therapy, 512–513
  - structure of, 510*f*
  - sulfamethoxazole with, 607
  - sulfonamides with, 607
- Trimipramine, 191
- Trinitrotoluene, toxicity of, 724, 738*t*
- Tripartite transfections, in gene therapy, 767
- Tripelennamine, 652*f*
- Triplet-repeat disorders, gene therapy for, 762–763, 763*t*
- Triptans, 241–242
- Trophozoites, of *Entamoeba histolytica,* 594, 594*f*
- Tropomyosin, of myocytes, 286, 288*f*, 289*t*
- Troponins, of myocytes, 286–289, 288*f*, 289*t*, 291
- TRPV1 and TRPV2 thermosensitive channels, 230–231, 231*f*
- Trypanosomiasis, drugs for, 596, 601
- Trypsin, therapy with, 776
- Tryptophan, in serotonin synthesis, 84*f*, 182, 182*f*
- Tryptophan hydroxylase, in serotonin synthesis, 180, 181*f*, 182, 182*f*
- TSH (thyroid-stimulating hormone, thyrotropin), 404, 408, 418, 419*f*, 779
- TsIg (thyroid-stimulating immunoglobulin), 418
- T-tubule, of myocytes, 287*f*
- T-type calcium ion channels
  - in absence seizure, 197, 199*f*
  - antiepileptic drug effects on, 201, 203
- Tuberculosis
  - drugs for, 571, 575, 578
    - combination, 606
  - *Mycobacterium tuberculosis* cell wall in, 569, 571*f*
  - purified protein derivative test for, 761
  - reactivation of, from tumor necrosis factor α inhibitors, 676
- Tubero-infundibular pathway, dopaminergic neurons in, 166
- Tuberomamillary nucleus, 79*t*, 80
- d-Tubocurarine, 101, 105, 221, 228
- Tubule(s), renal
  - drug excretion and reabsorption in, 37, 37*f*
  - in volume regulation
    - distal convoluted, 304*f*, 306, 306*f*
    - proximal, 304*f*, 305, 305*f*
- Tubulin
  - drugs acting on, 13, 530–531, 530*f*, 534
  - physiology of, 521, 523, 523*f*, 524*f*
- Tubuloglomerular feedback, in volume regulation, 301
- *Tumor,* as classic sign of inflammation, 617
- Tumor(s), malignant. *See* Cancer
- Tumor lysis syndrome, 514, 714
- Tumor necrosis factor α, 624
  - inhibitors of, for immunosuppression, 675–676, 675*f*, 681
  - release of, in amphotericin B therapy, 583
- Tumor promoters, 728
- Tumor suppressor genes, 728
- Turbulence, of blood flow, thrombosis in, 343, 343*f*

12-step programs, 260, 261b
Type I topoisomerase, in DNA replication and coiling, 521, 522f
Type II topoisomerase, in DNA replication and coiling, 521, 522f
Typical antipsychotic drugs, 171–174, 173f, 177
Tyramine, 113, 163
  toxicity of, in MAOI therapy, 188
Tyrosine, in catecholamine synthesis, 81, 84f, 108, 108f, 162, 162f, 181f
Tyrosine hydroxylase, in catecholamine synthesis, 162, 162f, 181f
Tyrosine kinase-associated receptors, 11, 12f
Tyrosine kinases, receptor, 10, 12f
Tyrosine phosphatases, receptor, 11, 12f

**U**

U fibers, seizure spread through, 196
Ubiquinone
  inhibitors of, 593
  in malaria parasite metabolism, 590, 590f
UDP (uridine diphosphate), in murein synthesis, 567, 568f
UDP-glucuronyl transferase, in drug metabolism, 49
Ulcers, peptic. *See* Peptic ulcer disease
Ultimate carcinogens, 727, 727f
Ultralente insulin, 466t, 467, 470
Ultrasound, for drug delivery from polymers, 786
Unasyn, 574
Uncoating, in viral life cycle, 546, 547f, 548f
Uncompetitive antagonists, 7
Underfill theory, of sodium retention, 308–309, 309f
Unexcretable drugs, definition of, 45
Unfractionated heparin, 349, 349f, 350, 355
Unidirectional block, re-entry in, 273, 274f
Unique drug targets, 494, 494t
Unitary hypothesis, of general anesthetic action, 221–222, 222f
Unstable angina, 326
  clinical management of, 389–390
  pathophysiology of, 385, 385f, 386
Uptake, prevention of, in drug resistance, 502
Uptake model, of anesthetic uptake and distribution, 210–218
  in abnormal states, 217.218t
  age effects on, 217,217f
  applications of, 215–218, 216f–218f, 218t
  cardiac output and, 216–217, 216f
  equilibration of alveolar with inspired partial pressure, 210–212, 212f
  equilibration of tissue with alveolar partial pressure, 212–213, 213t, 214f
  induction control and, 217–218, 218f, 218t
  overview of, 210, 211f
  rate-limiting step in, 214–215, 214f, 215f
  ventilation changes and, 216, 216f
Uracil
  in nucleotides, 506
  structure of, 507t, 513f
Urea breath test, in *Helicobacter pylori* infections, 686
Urease formation, in peptic ulcer disease, 686
Uric acid and urate
  drugs reducing, 713–715, 713f
  elimination of, 711
  formation of
    in gout, 711–712, 711t, 712f
    normal, 710–711, 710f
Uricase, for tumor lysis syndrome, 714
Uridine, structure of, 507t
Uridine diphosphate (UDP), in murein synthesis, 567, 568f
Uridylate
  in nucleotide synthesis, 510f
  structure of, 507t
  synthesis of, 507–508, 510f
Urine
  alkalinization of, for toxin elimination, 739
  drug excretion in, 36–38, 37f
Urofollitropin, 410, 414
Urokinase, 353, 356
Urticaria (hives), 650
Urushiol, 724, 738t
U.S. Patent and Trademark Office, brand name registration with, 798
Use patents, 801
Use-dependent block, by antiarrhythmic agents, 278

**V**

$V_1$ and $V_2$ receptors, in antidiuretic hormone action, 304
Vaccines
  for malignant melanoma, 768
  protein, 760–761, 779
Vacuolar hydrogen ion ATPase, in volume regulation, 305, 305f
Vagomimetic drugs, 101
Vagus nerve
  activation of, in asthma, 698, 698f
  parasympathetic fibers in, 73, 74f
Valacyclovir, 551f, 552, 562
Valdecoxib, 240, 244, 641, 641f, 644
Valganciclovir, 551f, 554, 562
Valproic acid
  action of, 200f
  for bipolar disorder, 185, 192
  for seizures, 201, 203
Valsartan
  for vascular tone regulation, 333
  for volume regulation, 311, 315
Valvular heart disease, heart failure in, 391
van der Waals forces, 5, 5t, 6f
Vancomycin, 568f, 572, 577
Vanilloid receptors (TRPV1 and TRPV2), 231, 231f
Vanillylmandelic acid, as norepinephrine metabolite, 110f
Vardenafil, 331
Variant angina
  clinical management of, 387
  pathophysiology of, 385, 385f
Varicella-zoster virus, drugs for, 547f, 551f, 552, 553, 553f
Vascular disease, protein-based therapy for, 764
Vascular endothelium, in vascular tone regulation, 320–322, 321f
Vascular permeability, chemical mediators in, 623t
Vascular rejection, acute, in transplantation, 669
Vascular resistance. *See* Systemic vascular resistance (SVR)
Vascular resistance-based hypertension, 378
Vascular tone, 317–333
  abnormal, in endothelial cell dysfunction, 385–386
  capacitance in, 318–322, 318f, 319t
  modulators of, 322–333. *See also* Vasoconstriction and vasoconstrictors; Vasodilation and vasodilators
    action of, 318–320, 319f, 320f
    α-adrenergic antagonists, 329, 333
    β-adrenergic antagonists, 329, 333
    angiotensin receptor antagonists, 333
    angiotensin-converting enzyme inhibitors, 333
    calcium channel blockers, 327–328, 328f, 328t, 332, 381
    case study of, 317–318
    hydralazine, 329, 333
    for hypertension, 381–382
    nitrates, 322–326, 324f, 325f, 331
    phosphodiesterase inhibitors, 326–327, 331
    potassium ion channel openers, 328–329, 332, 381
    renin–angiotensin system blockers, 330
    sites of action of, 322, 323f
    sodium nitroprusside, 324, 324f, 325f, 331
  regulation of, 320–322, 321f, 649
  resistance in, 318–322, 318f, 319t
  smooth muscle contraction and relaxation mechanisms in, 318–320, 319f, 320f
Vasoconstriction and vasoconstrictors
  endothelin in, 321–322, 321f
  in hemostasis, 336, 337f
  histamine in, 648–649, 648t
  with local anesthetics, 130–131
  mechanisms of, 319
  prostaglandins in, 630f
  renin in, 301–303, 302f
  thromboxanes in, 630f, 631, 632f
Vasodilation and vasodilators
  α-adrenergic antagonists, 329, 333
  β-adrenergic antagonists, 329, 333
  angiotensin receptor antagonists, 333
  angiotensin-converting enzyme inhibitors, 333
  calcium channel blockers, 327–328, 328f, 328t, 332
  case study of, 317–318
  chemical mediators, 623t
  for congestive heart failure, 398
  definition of, 319–320
  direct, definition of, 322
  histamine, 648–649, 648t
  hydralazine, 329, 333
  in inflammatory response, 624
  nitrates, 322–326, 324f, 325f, 331
  phosphodiesterase inhibitors, 326–327, 331
  potassium ion channel openers, 328–329, 332
  prostaglandins, 630f
  renin–angiotensin system blockers, 330
  sites of action of, 322, 323f
  sodium nitroprusside, 324, 324f, 325f, 331
Vasopressin. *See* Antidiuretic hormone (ADH, vasopressin)
Vasospastic angina, calcium channel blockers for, 388
$V_d$ (volume of distribution), 33–34, 33t, 34f
  half-life and, 39, 39t
  of local anesthetics, 131
Vecuronium, 101, 105, 228
Venlafaxine, 189, 191
Venodilators, for congestive heart failure, 396, 396f
Venous capacitance, in vascular tone, 318, 319f
Venous tone, 318, 319f
Ventilation, anesthetic delivery and, 216, 216f
Ventilation-limited anesthetics, 214, 216, 216f
Ventilation/perfusion mismatch, anesthesia induction in, 216
Ventral horns, 73, 77, 77f
Ventral tegmental area (VTA), 80f
  dopamine receptors in, 164, 165f
  dopaminergic neurons in, 166, 166f
  in reward pathway, 251, 257f, 258f
Ventricle(s)
  fourth, dopaminergic neurons in, 166
  left, pressure-volume loop for, 392, 392f
  myocytes of, electrical physiology of, 268–270, 269f, 270t, 271f

Ventricular depolarization, QRS complex representing, 271*b*, 272*f*
Ventricular fibrillation, 267, 276*b*
Ventricular repolarization, T wave representing, 271*b*, 272*f*
Ventricular tachycardia, 276*b*
Ventricular wall stress, in myocardial oxygen demand, 318, 319*f*
Verapamil
  for arrhythmias, 281
  for coronary artery disease, 388
  for hypertension, 381
  for vascular tone regulation, 327–328, 328*t*, 332
Vertical transmission, of drug resistance, 501
Very low-density lipoproteins. *See* Lipoprotein(s), very low-density (VLDL)
Vesamicol, 90, 91*f*, 98, 104
Vesicles, synaptic, regulation of, 67–68, 68*f*
Vesicular monoamine transporter (VMAT), 109, 109*f*, 112–113, 163*f*, 181*f*, 182, 182*f*
Vesicular stomatitis virus envelope protein, in gene therapy, 767
Vesnarinone, for contractility dysfunction, 297
Vessel poor group (VPG), in uptake model, 210, 211*f*
Vessel rich group (VRG), in uptake model, 210, 211*f*, 212, 213*t*
Vidarabine, 562
Vinblastine, 530, 534, 612, 613*f*
Vinca alkaloids, 530, 530*f*, 534, 610*t*
Vincristine, 530, 534
Virchow's triad, 343, 343*f*
Virilization, androgens for, 442
Virions, 546, 548*f*
Virus(es). *See also* Antiviral drugs; *specific viruses*
  drug targets in, 496–498, 497*f*
  for gene therapy. *See* Gene therapy, viral vectors for
  life cycle of, 497, 497*f*, 546–547, 547*f*, 548*f*
Vitamin A, teratogenicity of, 728
Vitamin $B_3$ (niacin), for dyslipidemia, 370–371, 371*f*, 373
Vitamin D
  in calcium homeostasis, 477–479, 477*t*, 478*f*
  deficiency of
    in kidney failure, 483, 483*f*
    rickets in, 479–480
  supplementation with, for bone disorders, 486, 489
Vitamin D-dependent rickets, 480, 480*t*
Vitamin K
  for anticoagulant poisoning, 742–743
  mechanism of action of, 350–352, 351*f*
VLDL. *See* Lipoprotein(s), very low-density (VLDL)
VM-26 (teniposide), 529, 533
VMAT (vesicular monoamine transporter), 109, 109*f*, 112–113, 163*f*, 181*f*, 182, 182*f*
Voglibose, for diabetes mellitus, 470
Voltage, in Ohm's law, 60, 60*f*
Voltage-dependent functions, 59
Voltage-gated calcium ion channels
  L-type, in vascular tone regulation, 319, 320*f*
  N-type, in pain transmission, 232, 233*f*
Voltage-gated ion channels, 7, 8*t*
  in action potential, 65, 65*f*
  in pain transmission, 128, 129*f*, 129*t*, 130*f*, 232
Volume capacity of tissue, for inhaled anesthetics, 212–213, 213*t*
Volume contraction alkalosis, 312
Volume depletion, perceived, in congestive heart failure, 307–308, 308*f*
Volume of distribution ($V_d$), 33–34, 33*t*, 34*f*
  half-life and, 39, 39*t*
  of local anesthetics, 131
Volume regulation, 299–316
  case study of, 299–300
  in cirrhosis, 308–309, 309*f*
  in congestive heart failure, 307–308, 308*f*
  disorders of, hypertension in, 378
  modulators of, 309–316
    antidiuretic hormone antagonists, 311
    for hypertension, 378–380
    natriuretic peptides, 311
    renal sodium reabsorption inhibitors, 311–316
    renin–angiotensin system inhibitors, 309–311, 310*f*, 315
    volume signals, 309–311, 310*f*
  in nephrotic syndrome, 309
  pathophysiology of, 307–309, 308*f*, 309*f*
  physiology of, 300–307
    intravascular determinants, 300, 301*f*
    renal sodium excretion control, 304–307, 304*f*–307*f*
    volume sensors, 300–301
    volume signals, 301–304, 302*f*, 303*f*
Volume-based hypertension, 378
von Willebrand factor, in hemostasis, 336, 337*f*, 338*f*
Voriconazole, 581, 581*f*, 582
VP-16 (etoposide), 529, 533
VPG (vessel poor group), in uptake model, 210, 211*f*
VRG (vessel rich group), in uptake model, 210, 211*f*, 212, 213*t*
VTA (ventral tegmental area), 80*f*
  dopamine receptors in, 164, 165*f*
  dopaminergic neurons in, 166, 166*f*
  in reward pathway, 251, 257*f*, 258*f*
VX nerve gas, 724, 725*f*, 738*t*

**W**

Wall stress
  in congestive heart failure, 394
  in myocardial oxygen demand, 318, 319*f*
Warfarin, 350–353, 351*f*, 352*t*, 355
  drug interactions with, 52*b*, 53, 352–353, 352*t*
  enantiomers of, 5
  toxicity of, 738*t*
Water
  body, regulation of. *See* Volume regulation
  protein affinity for, 5
  retention of, hypertension in, 378
Western blot test, for HIV, 761, 779
Wheal-and-flare response
  histamine in, 649
  in toxic exposure, 723
White blood cells. *See also specific cells*
  production of, 659–661
White matter
  brainstem, 77
  cerebral, 75
  spinal cord, 77, 77*f*
Withdrawal, of harmful drugs, 800, 800*t*
Withdrawal symptoms, 251
  addiction after, 253
  amphetamines, 258–259
  cannabis, 259
  cocaine, 258–259
  definition of, 249–250
  in detoxification program, 259–260, 260*f*
  digoxin, 294
  glucocorticoids, 433
Wolff–Chaikoff effects, 430
Worms (helminths), 587, 597–599, 597*f*, 602

**X**

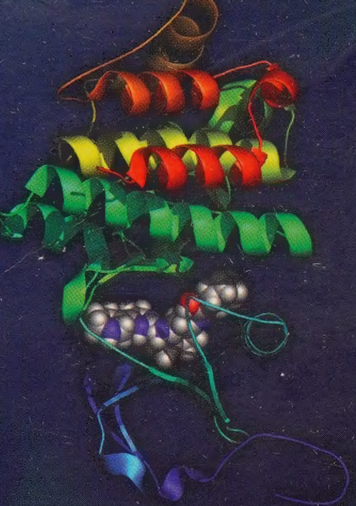
Xanthine oxidase, in purine metabolism, 710, 710*f*
  inhibitors of, 710*f*, 713–714, 713*f*, 715
Xenobiotics, definition of, 45, 719
X-linked disorders, gene therapy for, 762–763, 763*t*
X-ray crystallography
  in drug development, 754
  in drug structural analysis, 751

**Y**

Yeasts. *See also* Antifungal drugs; Fungi and fungal infections
  definition of, 579
Yohimbine, 116, 119

**Z**

Z lines, of myocytes, 287*f*
Zafirlukast, 634*f*, 642*f*, 643, 646, 702*f*, 703, 707
Zalcitabine (ddC), 551*f*, 563
Zanamivir, 547*f*, 559, 559*f*, 564
Zero-order kinetics, in clearance, 39
Zidovudine (AZT), 547*f*, 551*f*, 554–555, 555*t*, 556*b*, 563, 608
Zileuton, 634*f*, 642, 642*f*, 646, 805, 702–703, 702*f*
Ziprasidone, 174–175, 178
Zolendronate, for bone disorders, 485, 488
Zollinger–Ellison syndrome, peptic ulcer disease in, 687
Zolmitriptan, 241, 245
Zona fasciculata, 426, 426*f*
Zona glomerulosa, 426, 426*f*
Zona reticularis, 426, 426*f*
Zonisamide, for seizures, 204



## The first of its kind — a conceptual pharmacology text for medical, dental, and pharmacy students

Unlike other pharmacology texts organized by drug class, *Principles of Pharmacology* integrates relevant knowledge from the basic biomedical sciences — physiology, pathophysiology, cell and molecular biology, biochemistry, anatomy, neurobiology, microbiology, and immunology — to build an integrated, conceptual understanding of drug therapy. By discussing the therapeutic and adverse actions of drugs in the framework of the drug's mechanism of action, the book enables students to achieve a level of mastery in pharmacology that far surpasses that achieved by rote memorization. Content reflects recent advances in biological and biomedical science to improve understanding of mechanisms of drug action at the molecular level. Written and edited by Harvard Medical School students and faculty, the text blends the enthusiasm and perspective of student authors with the experience and expertise of faculty authors, to provide a comprehensive and consistent presentation of modern, mechanism-based pharmacology.

**HIGHLIGHTS AND FEATURES:**

- Each chapter begins with a clinical case specific to a target system, followed by the relevant biochemistry, physiology, and pathophysiology of the system. Each chapter presents and analyzes drugs and drug classes that affect the system by interacting with specific molecular and cellular targets.
- More than 400 two-color figures illustrate the molecular, cellular, biochemical, physiologic, and pathophysiologic processes underlying disease and depict the targets of drug therapy.
- Each chapter includes summary tables of drugs by mechanism, class, side effects, interactions, and contraindications, allowing for effective and rapid review.
- Contains chapters on important new areas in pharmacology and therapeutics, including how new drugs are discovered, drug delivery mechanisms, pharmacogenomics, protein-based and gene therapies, toxicology, integrative cardiovascular pharmacology, and integrative inflammation pharmacology.
- Use of up-to-date molecular and cellular science allows for the incorporation of new drugs into the learner's knowledge base.

"*Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*, a collaborative project of Harvard Medical School Faculty and Students, represents a refreshing and innovative departure in the preparation of a medical text . . . I predict with confidence that *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy* will play a vital role in the teaching and learning of pharmacology. Drs. Golan and Tashjian and their colleagues – both students and faculty – have made a significant and unique contribution in preparing this important book. Future generations of teachers and students, ar
mately the patients they will serve, will be indebted to them for this important contribution."

Eugene Braunwald, MD
Distinguished Hersey Professor of Medicine
Harvard Medical School
Boston, Massachusetts

ISBN 07817-4678-7
900
9 780781 746786
LWW.com